

The University of Chicago

A STUDY OF SOME EFFECTS
OF SEX HORMONES UPON THE EM-
BRYONIC REPRODUCTIVE SYSTEM
OF THE WHITE PEKIN DUCK

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
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By
LILLIAN BURWELL LEWIS

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I. Introduction	283
II. Materials and methods	284
III. Normal development	285
A. Development of the ovaries	285
B. Development of the testes	287
C. Müllerian duct development	290
IV. Experimental	291
A. Results obtained with Progynon	291
1. Males	292
Group I—dosage 0.25 mg.	292
Group II—dosage 0.50 mg.	296
Group III—dosage 0.75 mg.	300
Group IV—dosage 1.00 mg.	300
Group V—dosage 1.50 mg.	301
2. Females	302
Group VI—dosage 0.25—1.50 mg.	302
B. Results obtained with stilbestrol	303
C. Results obtained with hexestrol	305
1. Males	305
Group I—dosage 1.25 mg.	305
Group II—dosage 2.50 mg.	307
Group III—dosage 3.75 mg.	311
2. Females	311
Group IV—dosage 1.25—2.50 mg.	311
D. Results obtained with testosterone propionate	312
E. Results of histological study of sexual ducts	313
V. Discussion	316
VI. Summary	324
Literature cited	326

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I. INTRODUCTION

SINCE Lillie (1917) made the statement, at the conclusion of his now famous classic on the freemartin, that sex differentiation was controllable within variable limits in certain groups and that to determine the means, limits, and subsequent results of such control was one of the most important tasks of biology, many investigators have delved into the mysteries of experimental sex control. Dr. Lillie asserts in this paper:

In the case of the free-martin we do not find that hormones cause the development of any structure which is not represented embryologically in the normal female; the hormones act in this case merely by inhibition or stimulation of normal embryonic rudiments. If this should hold as a principle also on the male side we could not expect that the transformation of a testis into an ovary should ever occur, although the suppression of complete testicular development would probably happen.

In all the years that have elapsed since this investigation was made, very few exceptions have been found to the first part of the above statement, namely, that hormones cannot cause the development of any structure not embryologically present in the normal female; however, in several classes of vertebrates, a biological basis for a transformation in the male to the female direction has been found. Risley (1933) in the class Reptilia found that in the male musk turtle the gonads had both cortical and medullary components, demonstrating potential development in the female direction. Risley also has observed (unpublished data) that the alligator testis attains morphological sex differentiation without the complete loss of cortex (Forbes, 1938, p. 350). Among birds, Laulanié (1886) recorded the presence of a potential cortex on the developing left testis in the male chick, which was regarded by Swift (1916) as of historic interest only; hence

it passed into obscurity until Burwell (1931) observed such a potential cortex in the duck. Other birds are known to have this structure also; among them are the sparrow (Witschi, 1935) and the robin and the tern (Willier, 1939). In several mammals—the cat, dog, squirrel, guinea pig, mouse, rat, pig, sheep, calf, and man—Hett (1930, 1932) records the presence of a persisting germinal epithelium on the male gonads. This recital by no means exhausts the list and has not taken into consideration the status of the Anamniotes, wherein there are certain forms, such as *Bufo vulgaris* and *Rana temporaria* (Witschi, 1929) and others, that undergo a spontaneous sex reversal, indicating that these individuals are potentially bisexual.

With the discovery of a potential testicular cortex in the males of these various groups and the isolation and increasing availability of sex hormones in pure form, efforts to duplicate the "freemartin effect" were intensified, the use of hormone preparations largely replacing the older grafting experiments.

It was hoped that the effect of hormones upon the persisting germinal epithelium observed on the left testis of the duck embryo (Burwell, 1931) could be ascertained immediately, but uncontrollable circumstances delayed this work for a number of years. Meanwhile, it was established for the chick that this thickened germinal epithelium could be induced by homologous hormones to develop into cortex which brought about an intersexual condition or even complete inversion of the left testis (Kozelka and Gallagher, 1934; Wolff and Ginglinger, 1935a; Dantchakoff, 1936; Willier, Gallagher, and Koch, 1937).

In view of these important findings in the chick, it was decided to determine to what extent the response of the urino-

genital system of the duck to hormone treatment corresponds to the behavior of that system in the chick. The purpose of this investigation, therefore, was to ascertain (1) the normal behavior of the persisting germinal epithelium and the period of its duration in the duck; (2) whether complete inversion of the testes is possible by treatment with estrogens; (3) how late in the incubation period the germinal epithelium is modifiable by hormones; and (4) the effect of androgens upon the urinogenital system.

II. MATERIALS AND METHODS

The White Pekin duck was used throughout this investigation. Eggs were obtained from a farm in Indiana and a hatchery in Ohio.² The majority of these were shipped via express in cartons designed for duck-egg transportation and, with the exception of one lot, showed no evidence of improper handling in transit. As a rule, the eggs were unpacked and placed on trays in a cool basement, where they were allowed to stand for 24 hours, or overnight, before being placed in the incubator; however, toward the close of the experiments we were informed³ that this practice was no longer followed by many hatcheries, and thereafter the eggs were usually placed in the incubator upon arrival.

The incubators were electrically heated, thermostatically controlled, and provided with moisture by means of a sand tray. Moisture was not added prior to the 4th day of incubation, in order to permit a certain amount of evaporation and thereby obviate the danger of hormone's oozing through the perforation when the

eggs were injected. Only rarely did any exudation of fluid occur when this procedure was followed. When exudation did occur, the egg was slightly moved from side to side for a few seconds, during which time the exudate was usually with drawn into the opening. From the 4th to the 15th day the sand was kept fairly moist, and the eggs were sprinkled daily after the 10th day with water heated to 37°-39° C., as suggested by Rolnik (1943). After the 15th day the humidity was increased by adding additional water, and during the hatching period an even greater amount of water was added.

The temperature was kept between 101° and 102° F., and an attempt was made to subject the eggs to a fluctuating temperature, such as they undergo under natural conditions. This was accomplished by cooling the eggs at least twice a day after the first 24 hours for 5-minute periods, and after the 15th day the cooling period was prolonged to approximately 10 minutes. The eggs were turned at each cooling period.

In order to avoid waste of hormone, injections were not made until fertility was determined. Candlings were subsequently made at regular intervals in order to remove dead embryos.

For the injections, instruments designed and previously used by Dr. Domm were employed, and methods devised by him in his experiments on chicks were used. A 1-cc. hypodermic syringe with needle shortened to a length of approximately 3.0 mm. was employed. To puncture the shell and underlying membranes a pin vise, with inserted pin similarly shortened, was used. This precaution was taken in order to prevent injury to the embryo and surrounding vessels. All instruments were washed and sterilized immediately preceding and following injections and were kept between the

² Mrs. Blanche Tormohlen, Portland, Indiana, and the Twentieth Century Hatchery, New Washington, Ohio.

³ By Dr. H. J. Sloan, Department of Agriculture, Poultry Section, University of Minnesota.

folds of a clean towel while the eggs were being handled. Each egg was candled just before injection, to determine the location of the embryo, and a spot was marked on the shell for the aperture in close proximity to the embryo, care being exercised to avoid the large extra-embryonic blood vessels. The site of injection on the shell was sterilized by wiping with a small wad of cotton moistened in 70 per cent alcohol. The egg was placed in a small cardboard box containing cotton to hold it in position while it was being injected. The hormone was always deposited within a radius of about 0.5 cm. from the embryo. Injection age of embryos ranged from 3 to 18 days' in-

changes in the gonads or ducts, and many gonads and some ducts were sectioned for evidence of histological change (see Tables 3, 4, 7, and 8).

Sesame oil, the hormone solvent, was injected into 20 control eggs in the same manner and amount as the hormone solutions. The other controls received the same manipulation as experimentals but were allowed to develop untreated.

During the experimental period, a total of 2,793 eggs was handled. Treatment accorded these is shown in Table 1.

In the course of the investigation three estrogens and one androgen were employed. The estrogens were alpha-estradiol benzoate (Progynon-B), di-

TABLE 1
SUMMARY OF DATA ON TREATMENT OF EGGS

Dead or Infertile	Untreated	Sesame Oil	Progynon	Hex-estrol	Stilbestrol	Testosterone	Total
806	389	20	928	540	45	65	2,793

cubation. (Table 2, 1st col.). The small puncture was sealed by applying melted rubber paraffin.

A $\frac{1}{20}$ - or $\frac{1}{10}$ -cc. solution of hormone of varying concentration was injected at a time, and the egg was returned to the incubator with the sealed aperture turned slightly from the topmost position so that the embryo would remain close to the introduced hormone but, at the same time, not directly under the sealed puncture. This procedure reduced adhesions and consequent death of the embryos. Some of the embryos received a second and some a third injection on subsequent days (see Table 2).

The time of killing of the experimental birds ranged from 10 days' incubation to 24 days' post-hatching. All of these were examined under a binocular dissecting microscope to note and record any

ethylstilbestrol, and hexestrol. The androgen used was testosterone propionate.

III. NORMAL DEVELOPMENT

In an earlier study (Burwell, 1931) the development of the duck gonad was traced from its incipency as germinal epithelium through the 9th day, when sex differentiation had occurred. Up to that time the accessory ducts showed little or no differentiation. In the present investigation the condition of the reproductive system was studied, both macroscopically and microscopically, from the 10th day of incubation through the 24th day after hatching.

A. DEVELOPMENT OF THE OVARIES

Directing attention now to the gonads, we find that those of the female present the asymmetry characteristic of

most birds (Domm, 1927, and others). A macroscopic inspection revealed some variation in size of the right rudimentary gonad in duck embryos of the same age; yet in all cases examined it was readily observed, and in some instances the rudiment was quite prominent.

Many of the female gonads of the normal series were not prepared for microscopic study, but a fair sampling of from 10 to 26 days of incubation was studied to obtain an idea of the normal condition for comparison with experimental individuals.

1. *Left ovary*.—At 9 days' incubation, shortly after the time of sex differentiation, the germinal epithelium on the left ovary consists of several layers of cells containing scattered primordial germ cells with an underlying compact medullary tissue (Burwell, 1931). The picture has changed considerably by the 10th day. A decided line of demarcation, filled with a filmy network of connective tissue, has appeared between the epithelium and the medulla; and a sharp increase in the number of primordial germ cells has occurred, with definite beginnings of cortical cords, which cause this layer to be thicker than on the previous day. There seem to be very few primordial germ cells in the medullary portion of the left ovary, in contrast to the condition found in the right.

On the 12th day the cortex on the left ovary has increased in thickness and is composed of numerous cords. The germ cells have decreased somewhat in size and the area of filmy tissue between cortex and medulla is not so wide in extent. In the 14-day stage the left ovarian cortex is more compact in appearance, and the cells composing it are very uniform in character, instead of being grouped as definitely in cords as in the previous stage. The thinned-out area between

cortex and medulla is reduced in width; also very occasionally a group of differentiated cells, characteristic of the fully differentiated cortex, is found. These cells, which have been used as a criterion for differentiated cortex, are recognized by their large, deeply staining nuclei, with their compact mass of chromatin near the center of the cells surrounded by a clear area of cytoplasm, giving the cortex a much lighter appearance than the rest of the gonad (see Fig. 17). These differentiated cells are in the stage of syn- zesis.

At 18 days the patches of differentiated cells in the cortex of the left ovary have increased in number, so that the entire cortex, with the exception of the lateral margins and the anterior and posterior tips, has been transformed. By the 22d day, differentiation of cells in the cortical layer has extended to practically the anterior and posterior tips of the left ovary; but the lateral margins still show a border of undifferentiated cells on either side of the point of attachment, as seen in cross-section. This condition in the left ovary of a 22-day embryo is illustrated in Figure 16. On the 26th day the undifferentiated cell area mentioned for the 22d day usually has been transformed entirely into differentiated cells.

2. *Right rudimentary gonad*.—At 9 days' incubation the germinal epithelium on the right ovary is comprised of one or sometimes two layers, with numerous spaces between it and the underlying medulla. By the 10th day the right ovary is covered with a single layer of epithelium, which contains scattered primordial germ cells and in some cases a few large cortical cords that push out into the filmy area between the epithelium and the underlying medullary portion. Numerous germ cells; single or contained in cords, are found in the interior

of the gonad. Some germ cells lie very deep, so that the gonad in some cases is not separated into cortex and medulla as in the left one and only the single-layered epithelium stands out clearly from the rest.

On the 12th day the cells comprising the epithelium covering the right gonad have flattened, making this layer even thinner than on the 10th day. In some cases lumps of cortical tissue are found on the surface or, in others, scattered in the deeper portion of the ovary. At 14 days there is a heterogeneous grouping of lumps, which appear to be cortical in nature, and medullary tissue with occasional spaces which indicate degeneration. The lumps attached to the epithelium in some sections are reminiscent of the thickened condition of the epithelium found in sections of the left testis in untreated males of comparable age.

Although the right rudimentary gonad does not exhibit well-defined cortex and medulla at the 18th-day stage, there may be found among the cells which constitute its bulk some scattered cells resembling those in synizesis in the differentiated cortex of the left ovary. These are believed to be derived from the primordial germ cells, which were seen in the medullary tissue in earlier stages; and they form, in certain instances, cords that contain numerous cells—all in synizesis—that appear no different from cortical cords with cells in synizesis in the cortex. In some treated cases, even the medulla of the left ovary contains cords of these cells in synizesis that seem the same as those in its cortex. The bulk of the right ovary, therefore, in many instances displays a mixture of medulla and cords with cells that appear to be descended from the primordial germ cells and are comparable in a large measure to cortical cords. On the 22d day the right

gonad may show, near the periphery, patches of cells in synizesis. One embryo of this age also shows a few cells of this appearance among the medullary cords, which are probably derived from the primordial germ cells.

A most interesting observation was made upon the right rudimentary gonad of one 26-day embryo. In addition to patches of cells in synizesis among the medullary cords, there was noted in the deepest portion of the gonad a few tubules which were decidedly testicular in appearance. In none of the nine untreated females studied up through the 26th day did we find a well-defined cortex; yet, on the other hand, there was no individual which failed to show some cortical lumps, either very scattered or fairly numerous.

Since the chief interest in the female was to ascertain the normal appearance of the ovary throughout its various stages of differentiation, histological study of normal females was carried no further than the 26th day of incubation.

B. DEVELOPMENT OF THE TESTES

While it has been known for some time that the ovary possessed the structural basis for sex reversal in the medulla (Lillie, 1927; Domm, 1927, 1929; and others), it was generally believed that the testis in higher forms lacked the homologue of the ovarian cortex. Laulanié (1886) noted a thickened germinal epithelium on the left testis of the chick, which persisted from the 7th to the 11th days of incubation, and he regarded this as homologous to the cortical layer of the ovary. Kozelka and Gallagher (1934) injected male human urine extracts—theelin and theelol—into hen's eggs and caused the development of ovotestes on the left side in male embryos. These results, therefore, clearly indicated the ability of this *couche ovigène* of Laulanié

to develop into ovarian cortex, although these investigators did not so interpret their findings. Similar phenomena have since been demonstrated by a number of other investigators. Other instances in which this persisting epithelium has been noted on the testis are Burwell (1931) in the duck; Hett (1932) in the cat, pig, sheep, calf, man, dog, squirrel, guinea pig, mouse, and rat; Risley (1933) in the musk turtle, also the alligator; Witschi (1935) in the sparrow, and Willier (1939) in the robin and tern.

1. *Left testis*.—On the 9th day of incubation, soon after the time of sex differentiation, the left gonad of the male duck embryo shows a well-defined germinal epithelium, consisting of cells with rounded nuclei separated from the sexual cords by a basement membrane (Burwell, 1931). The epithelium of this gonad shows an occasional germ cell at this stage. These cells gradually increase in number, and on the 12th day a germ cell here and there becomes multinucleate, causing the appearance of lumps on the margin of the gonad. The number of nuclei, which do not always have well-defined cell walls, varies from two to eight or more. These lumps increase in number and in size through the 14th and 16th days and in some instances appear to fuse to form a continuous layer. Figure 18 shows a 14-day embryo with lumps of various sizes developing from germ cells in the epithelium, while Figure 19, of a 16-day embryo, illustrates apparent fusion of these lumps on the gonad to form a continuous layer.

A maximum thickness appears to be attained on the 24th day, and male embryos of 14, 16, 18, 20, 22, and 24-days exhibit a condition of the left gonad which is indicative of the fact that the germinal epithelium proliferates cords of cells that form a rather imposing layer.

Figure 20 illustrates such a layer near the caudal end of a 24-day normal male, and Figure 21, from the same embryo, shows the nature of this layer about the mid-portion of the gonad. These new cords of cells differ from the old, in that the peritoneal nuclei have not arranged themselves on either side of the cords in a compact layer with germ cells toward the center, as the old cords have, but are scattered uniformly in a dense condition throughout the cords. The arrangement of the germ cells in the older cords is in agreement with the observations of Swift (1916), who noted that the germ cells were not confined to the center of the tubules in the chick. Semon (1887) and Popoff (1908) held that the germ cells in the chick were placed centrally within the tubules.

A total of seventeen male embryos, ranging in ages between 14 and 24 days, was examined, and all showed development of these cords in varying degrees, some slight but in most cases pronounced. There would seem to be no doubt that the epithelium is proliferating a second set of cords.

Grünwald (1934b) describes the development of secondary sexual cords, *Keimstränge*, in the human testis, which are analogous to the secondary sexual cords of the female. To his knowledge these had not been observed before. Although Hett (1930) records the presence of cords of testis epithelium, he does not trace their development into typical sexual cords. Grünwald did not see these cords in all embryos studied, and he had not determined at the time why they were sometimes absent; when they were noted, they were found on both testes. He observed that, as development proceeds, these cords make numerous unions with the primary cords and later cannot be distinguished from them.

In the case of the duck it appears that the large secondary cords become incorporated into the testis and lie intimately alongside the primary cords. Figure 19 shows the left testis of a 16-day untreated male embryo, which is suggestive of such a fate. This illustration is from an embryo 2 days older than that in Figure 18, which illustrates an earlier stage in the formation of the secondary cords. By continued development and more or less fusion, the result is a fairly continuous layer in apposition to the primary cords, as revealed in Figure 19. In the thirteen other embryos examined, with ages ranging from 16 through 24 days, at which time maximum thickness is reached, there was indication of varying degrees of fusion of cords to form an outermost layer. This layer in these cases might be composed entirely of separate cords, partially fused cords, or nearly completely fused cords.

Observation of actual connection between primary and secondary cords, as recorded by Grünwald for man, has not been noted in the duck, and it is not certain what the fate of these secondary cords is; but a 28-day-old embryo which shows a group of these newly formed cords inside the tunica albuginea, in close contact with the primary cords, is suggestive of a similar destiny (Fig. 22). After the 24th day of incubation the investing outer layer of newly formed cords becomes less obvious as such, as revealed by three embryos, 26 days old. One of these showed hardly a trace. In addition to the 28-day-old embryo already mentioned, four others of that age were examined, and one of these showed a marked degree of fusion of secondary tubules in contact with the primary tubules near the posterior end of the gonad, one possessed a few small lumps at the posterior end, and two exhibited a very thin

layer in places which contained some germ cells.

After hatching, the layer of secondary cords on the left gonad becomes more and more inconspicuous. This was shown by observations upon ducklings of 1, 2, 4, 6, and 8 days post-hatching. The latest age at which fairly large lumps were noticed in connection with the epithelium was 2 days. At 8 days, only an occasional flattened lump was found, which represents the remnants of the epithelium. In 12-, 16-, and 26-day-old post-hatch birds, these lumps became even more scarce, and the one male duck among the three killed on the 24th day showed no trace of them. Just a minute trace was found on only one of the two 20-day-old birds. It would seem from this that practically all traces of thickening in the epithelium vanish by the 24th day after hatching or shortly thereafter. Primordial germ cells in the secondary cords were noted last in a duck of 16 days' post-hatching.

2. *Right testis*.—At 9 days the cells composing the outer layer of the right testis are much flattened, by way of contrast to the rounded ones comprising the persisting germinal epithelium on the left testis, and are set off by an incipient tunica albuginea. As incubation proceeds, the area between the testicular cords and the epithelial layer widens, as a loosely organized connective tissue develops within it. By the 20th day the connective tissue has become more compact, containing many flattened nuclei; and on the 24th day these flattened nuclei have come into intimate contact with the outer epithelial layer, aiding in forming the tunica albuginea proper and leaving spaces intermittently between this structure and the testicular cords.

Once in a great while the right testis may display a feeble attempt to develop a germinal epithelium. This is manifested

in later stages by an occasional thickened lump, but in no instance was a well-developed germinal epithelium observed on the right testis, as was found in many instances on the left.

Multiplication of the tubules within the gonads produces on the surface of the gonad, in some instances, fine ridges or elevations. These are seen as early as 18 days' incubation and are not evident in all normal embryos. At the caudal end the proliferating tubules sometimes extend from the surface of both gonads onto the surface of the underlying mesonephros, producing small, flattened, finger-like processes, with the result that the gonad margin is not smooth and rounded but is diffuse in character.

By way of summary of testicular development, the persisting germinal epithelium covers the entire left gonad in a well-defined layer of more or less uniform thickness at 10 days' incubation. As development proceeds, this structure tends to become thinner and eventually disappears anteriorly while thickening posteriorly, the maximum amount of increase in depth being attained caudad of the mid-line of the gonad, where it appears that the layer may give rise to secondary sexual cords in some instances. There is a possibility that these secondary cords may be incorporated into the gonad along with the primary.

On the right gonad, vestiges of this layer are present from time to time; however, no continuous epithelium was found on this testis at any time. Since this epithelium is lacking to any marked degree, the reason is apparent why the right testis does not undergo inversion or partial inversion, as the left does when treated with estrogens. This gonad also apparently fails to proliferate secondary cords, as the left does normally.

C. MÜLLERIAN DUCTS IN THE MALE

The Müllerian ducts in the male appear to reach their maximum development about the 10th day, for in some 11-day males signs of degeneration are apparent. As was observed by Lillie (1919) in the case of the chick, retrogression begins posteriorly and proceeds anteriorly. There is a thinning of the thick-walled duct, which becomes more and more pronounced until a mere line indicates the original position, and then this line disappears. This process of degeneration extends from about the 11th to approximately the 18th day, when the vanishing-point has been attained. The extreme anterior tips have been noted to persist until as late as the 26th day. The period of retrogression appears to be of longer duration than that described by Lillie (*ibid.*) for the chick, in which it is stated that development is complete on the 8th day and degeneration is at an end or far advanced on the 11th day. Willier (1939) observes that disappearance is complete by the 18th day in typical cases.

D. MÜLLERIAN DUCTS IN THE FEMALE

In the female, degeneration of the right Müllerian duct proceeds in the reverse direction of that in the male, namely, from anterior to posterior end. In 12-day females the right duct is still entire and has the appearance of that of a 10-day male, whereas the left shows indications of differentiation into the definitive adult form. In females of 13 days, the anterior end of the Müllerian duct on the right side begins to recede, while differentiation on the left is more pronounced. By the 14th day, regression on the right has reached the posterior end of the Wolffian body, or approximately so. On the 16th day the anterior, or mid-level, of the shell gland has been attained, and

the 18th day reveals a shrunken vestigial tip adjoining the cloaca, which persists apparently indefinitely. The left duct has completed its differentiation at this stage. In neither male nor female did we observe an abnormal persistence of Müllerian ducts in normal individuals.

IV. EXPERIMENTAL

A. RESULTS OBTAINED WITH ALPHA-ESTRADIOL BENZOATE (PROGYNON-B)

In the use of this hormone there was a variability in the time of injection, the amount of hormone used, and the age at which the embryos were killed. These data, along with the number of eggs injected and survival percentages, are summarized in Table 2.

A total of 356 embryos was examined macroscopically to determine the effects of Progynon on the reproductive system. Of these, 181 were males and 175 females. The males were distinguished by less disparity in gonad size, macroscopically, and by the histological character of the primary cords. Of the 181 males, 124 were studied microscopically also. A glance at the survival percentage in Table 2 will reveal that the mortality rate is very high, especially just prior to hatching and late in the season. The dosages employed ranged from 0.25 to 1.5 mg. Because of the low degree of solubility of the hormone, 5 mg. per cubic centimeter, the quantity of material which would have had to be injected in order to increase the dosage precluded larger doses in the early stages. The largest dose, 1.5 mg., failed to bring about complete sex reversal of the testis, although a high degree of intersexuality was attained. Willier, Gallagher, and Koch (1937) found that 1.0 mg. of theelol was adequate to bring about complete reversal of the left testis of a zygotically determined male chick into an ovary;

Wolff and Ginglinger (1936a) effected complete reversal of the left testis in the chick with 0.05 mg. of folliculin; and Dantchakoff (1936), by using 2,500 mouse units of folliculin, was able to produce the same result. These investigators, however, used different methods of administering the hormones, which, no doubt, accounts for the differences in amounts of hormone required to bring about complete reversal.

On the basis of these comparisons it would seem that complete inversion of the testis is not possible in the duck or that larger quantities of Progynon are requisite to bring about this change in the chick than are required of other estrogens.

In view of the absence of complete inversion and the failure of Progynon to effect such profound changes in the duck as those described by other investigators in the case of the chick, it seemed best to discuss results from the standpoint of dosage, time of injection, and degree of modification at the time the embryos were killed. A study of the normal stages of gonad development in the female reveals that, in the 14-day embryo, synizesis may be observed in a few cortical cords. As development proceeds, this condition may be noticed in more and more cords, so that, by the 18th day, only anterior and posterior tips and the margins bordering the hilus are not in synizesis. This condition has been used as a criterion for fully differentiated cortex.

The earliest stage in which fully differentiated cortex was observed on the left gonad of the male after treatment with Progynon was at 22 days following injection on the 5th day (Case 4P1522 ♂ b). However, cases have been noted in which cortical cords are definitely recognizable as early as 14 days after treatment, the embryo being 18 days old (Case 13P1418

a♂). The very youngest individual in point of injection age to show incipient cortical cords was 13 days after injection and at 18 days' incubation (Case 8P2518♂b). With these facts in mind, the cases discussed in Groups I, II, III, IV, and V include only birds in which a minimum of 14 days had elapsed since treatment.

in synzesis. One of these was injected on the 3d day, one on the 5th, and two on the 8th. The reproductive system of the 3-day injected case was somewhat more highly modified than the 5-day injected embryo, as is shown in Figure 3. Comparison with the normal male and female systems, Figures 1 and 2, respectively, reveals the intersexual condition ex-

TABLE 2
DATA ON THE EFFECT OF PROGYNON

INJECTION AGE* (DAYS)	QUANTITY INJECTED (MG.)	NO. OF EGGS INJECTED	EMBRYOS KILLED				SURVIVAL (PER- AGE)
			Age*	No.	Male	Female	
3.....	0.25	14	18-26	14	5	5	71
4.....		30	22-26	21	6	10	53
5.....		94	10-26	72	12	17	30
8.....		33	22-26	21	8	8	48
3.....	0.50	18	26	6	2	0	11
4.....		182	16-26	120	32	30	34
5.....		90	14-26	78	14	7	23
6.....		52	14-26	40	13	17	56
10.....		54	16-8 †	54	17	11	51
12.....		83	18-2 †	67	20	22	50
14.....		48	20-8 †	49	16	17	62
16.....		22	22-28	18	3	11	72
18.....		34	22-27	30	11	8	52
4 and 6.....	0.75	28	20-26	14	4	5	32
4 and 6.....	1.00	31	20-26	15	9	2	35
4, 6, and 8.....	1.50	63 ‡	25	23 ‡	8	5	20
Totals of sexes					181	175	
Sex ratio (per cent).....					51	49	

* Incubation days.

† Post-hatching.

‡ The discrepancy in all cases in the table between number of eggs injected and embryos killed is due to the death of embryos in the interim.

1. *Males: Group I.*—The dosage in this instance was 0.25 mg., and the ages at the time of injection ranged from 3 to 8 days. A total of twenty-one male embryos was examined macroscopically and microscopically. Of these, nine were 26 days old and so had the longest period in which the hormone might exercise its influence. Four of the nine 26-day embryos had cells in the cortex which were

hibited by this embryo, which was clearly visible to the naked eye. These three figures represent embryos of the same age and are drawn to the same scale. The gonads are clearly of an intermediate type. They are more flattened than the male and more rounded than the female. The right is approximately the size of the left, but all along its lateral margin are detached lumps of testicular tissue.

In the left gonad, microscopic study discloses a thin surface layer of cortex, with many cells in synizesis but not uniformly distributed, extending over most of the gonad surface (Fig. 24). It does not extend down to the hilus over the medial or lateral border, or to the anterior tip. The layer becomes thinner and confined more to the medial margin as the anterior end is approached and finally disappears rather close to the tip. Posteriorly, there is a gradual increase in thickness; but the thicker, even layer gives way to a lumpy cortex, which appears less differentiated in character. These lumps extend to the posterior end, and some of them are continuous with outgrowing primary cords. The cortex for the most part, however, is cut off from the underlying testicular cords, which in many instances push up against the cortex or among its cords. There is little or no difference in character in these secondary sexual cords, which occupy a cortical position, and the normal testicular cords of untreated males.

On the right gonad there is no definite cortex but only an occasional flattened patch of cells outside the tunica albuginea. Medullary cords are not apparent in either gonad.

The Wolffian ducts in no instance show effects of Progynon administration. The Müllerian ducts in the case just described show almost typical female development, the exception being a disparity in shell-gland size in favor of the normal.

One of the two embryos injected on the 8th day showed as good, if not better, differentiation of cortex as did the 3d-day injected embryo. The other exhibited a very thin layer of differentiated cortex on the posterior portion. In both cases the Müllerian ducts had developed into cystlike structures at the anterior

ends, with no enlargement posteriorly. These cysts were filled with a watery fluid. For the gonad and duct condition of others in this and related lots see Table 3.

The next to be considered in this series of embryos that received 0.25 mg. of



FIG. 1.—Reproductive system of normal 26-day male embryo.

FIG. 2.—Reproductive system of normal 26-day female embryo.

Progynon ranged in age at killing from 18 to 24 days and were injected on the 3d, 4th, 5th, and 8th days. These were from the same lots as the above-described 26-day individuals but were removed at earlier ages. In each instance the minimum interval required for the appearance of cells in synizesis in the cortex had elapsed. Of the twelve cases in this unit, five showed development recognizable as cortex, two of which displayed some cortical cells in synizesis. The best cortex

here was considerably inferior to that described for the 26-day cases.

As to the Müllerian ducts of these twelve cases, only two instances were found in which either duct was entire. The persisting duct was the left, and the

the surface in both gonads. On the left gonad it is recognizable microscopically as cortex with patches of cells in synizesis; on the right there are thickened areas of epithelium, which are thinner than the cortex on the left gonad. The protocol re-

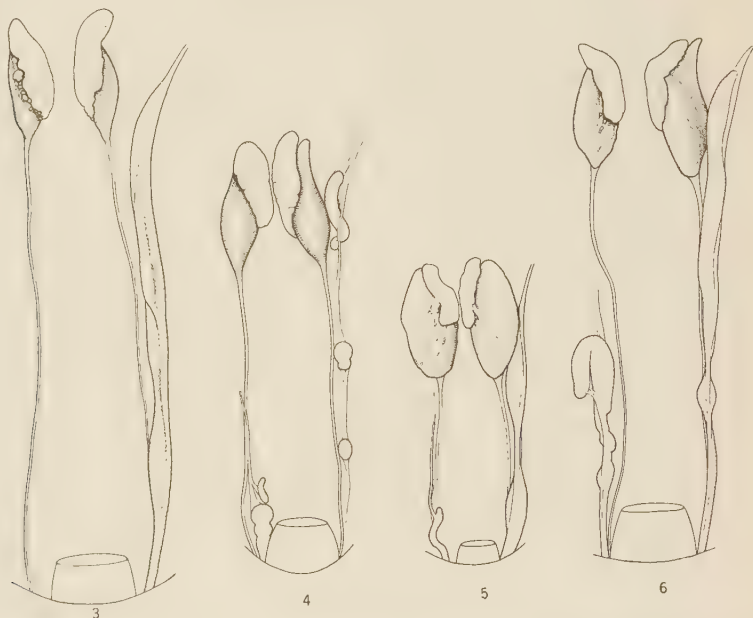


FIG. 3.—Reproductive system of genetic male embryo injected with 0.25 mg. Progynon on 3d day. Age 26 days. Note slightly flattened left gonad (ovotestis), detached lumps of right oviduct, and female-like left oviduct.

FIG. 4.—Reproductive system of genetic male embryo injected with 0.25 mg. Progynon on 4th day. Age 22 days. Note macroscopic evidence of cortical lumps on surface of gonads and the cystic character of the oviducts.

FIG. 5.—Reproductive system of genetic male embryo injected with 0.50 mg. Progynon on 4th day. Age 18 days. Note early modification of gonads and female-like oviducts.

FIG. 6.—Reproductive system of genetic male embryo treated with 0.50 mg. Progynon on 4th day. Age 24 days. Left gonad appears female-like and flattened, right is more rounded. Oviducts are of female type.

Müllerian duct development of these individuals was very much like the normal female. In the others the ducts were either absent, possessed of swollen cystic anterior tips, or had several cysts at intervals, all fluid-filled, as shown in Figure 4, which exhibits the second best-developed case in this group. This case is also interesting in that the thickened epithelium is indicated macroscopically on

veals that this embryo was 22 days old and had been injected on the 4th day.

As regards gonad surface, four other embryos displayed minute ridges over the entire gonad. In one case both gonads were involved; in the others the left gonad only. Microscopic examination disclosed that the sexual cords pushed through the forming tunica albuginea, causing the remaining single-celled layer

to buckle outward. Willier, Gallagher, and Koch (1937) record a somewhat similar phenomenon in the right testis of the chick. They state that the cords not only connect with the free gonad surface but appear to open into the coelom. This ridged condition has been noted in normal duck embryos, but it was not so exaggerated as in the experimental ones.

The youngest killed cases treated with 0.25 mg. of Progynon ranged in age from 10 to 18 days. In an embryo injected on

the 5th day, evidence of stimulation of the germinal epithelium was noted as early as the 10th day and was reflected in an increase in the number of germ cells over the normal and in the appearance of incipient cortical cords. Early development of these cords may be traced in embryos 12, 14, 16, and 18 days of age. They may be distributed uniformly or confined to definite spots which are discernible with the unaided eye on the surface of the gonad. Such localized cortical

TABLE 3

SUMMARY OF EFFECTS OF PROGYNON ON GONADS AND MÜLLERIAN DUCTS OF MALES

No. OF EMBRYOS	DOSAGE (MG.)	INJECTION AGE (DAYS)	INCUBATION AGE (DAYS)	GONADS			MÜLLERIAN DUCTS														
				Differentiated Cortex*	Undifferentiated Cortex	No Definite Cortex	Left Entire	Left Inc.†				Left Cystic‡	Right Inc.						Right Cystic	No Ducts	
								1/2		1/2			1/2		1/2		1/2				
								A.	P. ¶	A. P.	A. P.		A. P.	A. P.	A. P.	A. P.	A. P.	A. P.			
5.....	0.25	3	18-26	I	I	3	I	...	...	...	I	I	...	...	...	...	...	...	I	I	2
6.....		4	22-26	2	I	3	I	...	...	...	...	I	...	...	...	...	...	...	2	...	4
12.....		5	10-26	I		11	8**	I	...	...	I	I	...	...	I	...	I	2	I	I	
8.....		8	22-26	2	I	5	...	...	...	...	3	3	...	...	...	...	6	...	...	2	
2.....	0.50	3	26	I	I		I	I	...	...	...	...	...	...	...	...	...	2	...	...	
32.....		4	16-25	6	6	20††	14	I	...	2	4	I	5	...	...	2	I	15	5	5	
14.....		5	14-26	6	3	5	4**	I	...	...	3	3	...	...	3	I	...	2	3	3	
13.....		6	14-26	2	I	10††	2	I	...	2	5	3	...	...	4	2	2	2	2	I	
18.....		10	16-8††			18					5	2			I		4		5	7	
21.....		12	18-2†††			21					9	2				5		9	5	I	
16.....		14	20-8††			16					4	I				3		1	I	11	
3.....		16	22-24			3														3	
11.....		18	22-27			11					2								I	9	
4.....	0.75	4, 6	20-22		3	1††	I	...	...	...	2	I	...	I	...	I	...	I	...	...	
9.....	1.00	4, 6	20-26	I	7	1††	2	I	...	I	2	3	...	...	I	...	4	2	...	...	
8.....	1.50	4, 6, 8	25	3	2	3††	4	...	...	I	I	2	I	...	2	I	...	4	...	...	

* "Differentiated Cortex" refers to cortex with cells in synizesis, and they may be few or many. "Undifferentiated Cortex" indicates cortex lacking cells in synizesis.

† "Inc." = Incomplete.

‡ The term "Cystic" in this table refers to fluid-filled enlargements occurring singly or severally, provided they do not occur singly at either anterior or posterior extremity of the Müllerian duct. Cysts found there have been included in the fractional lengths of these respective ends.

§ The fractions indicate approximate lengths from either end.

|| "A." = Anterior end.

¶ "P." = Posterior end.

** Some too young to show degeneration.

†† Not all sectioned.

††† Post-hatch age in days.

areas are shown on the gonads in Figure 4. No fully differentiated cortex was observed in any of these ten cases which comprise the youngest category. Study of these earlier stages rules out any possibility of early cortical proliferation and subsequent degeneration prior to hatching.

Müllerian duct development ranged from no unusual development in the 10-day cases, through a regional development, similar to Figure 4, to a complete left duct with posterior vestigial tip on the right in an 18-day embryo. Those male embryos 10, 12, and 14 days old shown in Table 3 with entire left ducts display a duct condition typical of the normal young male embryo before degeneration sets in. The absence of degeneration in the 12- and 14-day-old cases, when regression is normally apparent, may result from individual differences in rate of development or maintenance by the introduced estrogen.

Group II.—Dosage here was 0.50 mg., and the injection age was from 3 to 18 days. In those embryos injected from the 10th through the 18th day this quantity of Progynon had no noticeable effect upon the germinal epithelium; but there was Müllerian duct hypertrophy after 10 days, so these cases will be included only in the discussion relative to Müllerian ducts. This leaves forty-eight male embryos injected between the 3d and 10th days for consideration, with an age range of from 18 to 26 days. Two embryos injected on the 3d day and killed on the 26th day showed sexual cords protruding individually and collectively from the surface of the left gonad. They may project alone or anastomose, forming a cap readily distinguished from the rest of the gonad. In this cap in one of the embryos, a layer of cells in synizesis increased to a depth of two cells in places.

The other did not show this differentiated layer but the cap of cords was definite. The condition really suggests the proliferation of a lump of cords on the surface of the gonad from underlying testis cords, which lump is endowed with the ability to form cortex. The surface of the left gonad in treated individuals frequently exhibits a pronounced corrugated appearance, which in some instances may be indicative of this condition.

In many cases observed in this investigation the character of the thickened outer layer on the left testis in embryos of from 20 to 26 days' incubation has the aspect of having been formed by outgrowing testicular cords rather than by cords formed in the epithelium. Figure 32 shows a cap of cortex with connecting testicular cords. When this cap is traced anteriorly, it covers a larger area of the gonad and shows cells in synizesis. Figure 31 illustrates an early stage in the outgrowth of testicular cords which form surface lumps.

Thirty male embryos were examined in the 4-day-old injection group, eighteen of them microscopically. These eighteen were sectioned because gross examination revealed more variation from the normal in gonad and Müllerian duct formation than in the others.

The effect of Progynon was strongly registered upon the gonads of this group in macroscopic appearance. The lateral margins of both right and left gonads were decidedly irregular in outline, showing flattened, finger-like projections, as described above, extending over the Wolffian body, which in some instances terminated in detached lumps; or there might be detached lumps along the lateral border occurring with greater frequency toward the posterior end (see Fig. 3). These projections or detached

lumps, when sectioned, proved to be testicular in character. The right gonad usually appeared broader from the ventral view and did not show a marked tendency to become rounded, testis-like, as observed by Willier *et al.* (1937) and others, in the chick. The left was always longer, as Figures 5 and 6 reveal; yet occasionally it was smaller in diameter. This disparity in length was approximately as much as one-third in some cases.

Only two of the eighteen embryos sectioned showed a marked differentiation of the cortex, well delimited from the underlying cords, which were testicular in nature. These were killed on the 25th day. The cortical tissue did not cover the entire ventral surface of the gonad in either case but was applied caplike, with both medial and lateral margins uncovered. It extended almost the length of the gonad also, but anterior and posterior tips were bare. In one case the maximum thickness approximated closely the normal of comparable age but was decidedly lacking in the extent of this thickness. Many of the cells in these two cases were in synizesis.

The other sixteen individuals displayed all the intermediate stages, from those just described to no noticeable stimulation. Some of these showed a thin layer, sometimes one cell in thickness, of cells in synizesis in places, while others showed a rather thick layer, not well marked off from the underlying tissue, which had the appearance of cortical tissue in the presynaptic stage. The ages at which this group was killed ranged from 18 to 25 days (see Table 3 for complete record). One case killed on the 18th day showed an early stage in cortex formation from the germinal epithelium (Fig. 23). The gross picture of the genitourinary system of this individual is shown in Figure 5.

Müllerian duct development was good in this lot. Figures 5 and 6 show hypertrophy throughout in the left duct, with a variable degree of hypertrophy on the right. Fourteen of the thirty embryos showed development of the left duct in its entirety, with the right persisting from more than half its length to not at all; five showed no Müllerian ducts. In the other cases the persisting parts were cystlike, which occurred singly or several to a duct in tandem fashion at any level. The higher dosage of hormone administered in Group II evidently accounts for the superior duct development over Group I.

The next lot under Group II was injected on the 5th and 6th day. Seventeen embryos comprise this group, and sixteen were sectioned for microscopic study. On the whole, cortex development was not so good here as in the embryos injected on the 4th day. Eight showed a fair degree of cortical differentiation, but all were inferior to the 4-day injected embryos in this regard. In four others, cortical differentiation had proceeded only far enough to be recognizable as such. The gross contour of the gonads did not appear so greatly altered either, as Figures 7 and 8 reveal. There is some irregularity along the margins, accompanied by detached lumps and a slight disparity in size but not so exaggerated as in the previous lot. Figure 8 shows a flattening of both gonads, giving an ovary-like appearance.

In five embryos of this category with the best-developed cortex, this tissue extended over most of the gonad surface, being thicker in all instances toward the caudal end. In places, especially cranial, it was not well delimited from the underlying tubules. All these embryos were 26 days old when killed, except one 24-day embryo, which had as thick a cortex but

not so many cells in synizesis. Three of these birds were injected on the 5th day and two on the 6th day. The 6-day cases were about as good as the 5-day. The other three embryos with differentiated cortex not so well developed had been injected on the 5th day and were 22 days

day. However, this is not absolutely conclusive evidence, although it suggests that Progynon may not be so effective when injected on the 5th or 6th days as when injected on the 4th, because there were many more cases to judge from in the 4-day injected category.

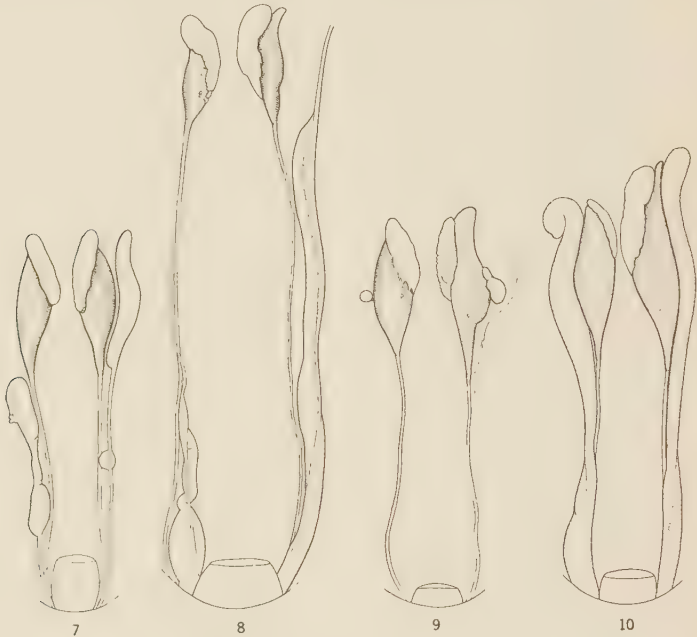


FIG. 7.—Reproductive system of genetic male embryo treated with 0.50 mg. Progynon on 5th day. Age 22 days. Note greater length of left gonad and character of oviducts.

FIG. 8.—Reproductive system of genetic male embryo treated with 0.50 mg. Progynon on 5th day. Age 26 days. This shows a good intersexual condition.

FIG. 9.—Reproductive system of genetic male embryo injected with 0.50 mg. Progynon on 12th day. Age 22 days. Note corrugated condition of left gonad, also persisting anterior ends of oviducts.

FIG. 10.—Reproductive system of genetic female embryo of same age and treatment as shown in Fig. 9. Contrast the well-developed right oviduct with those of the male.

old. The gonads represented in Figure 7 are from one of these 22-day-old embryos whose cortex was too poorly developed to be ranked among the first five. It consisted of a very thin layer, with a few differentiated cells here and there. Killing age might have been a responsible factor. On the whole, development of cortex did not appear to measure up to the standard of the embryos treated on the 4th

Müllerian duct development, on the whole, was good in the above lot. The left duct was entire and female-like; practically entire, as illustrated in Figure 8; or entire with enlargements connected by slender portions, as shown in Figure 7. There were seven of these. The size in the others ranged from these conditions to merely a persisting anterior tip. The best right Müllerian duct development is

also shown in Figures 7 and 8. In four specimens it was absent.

Ten other embryos injected on the 5th and 6th days, whose ages at killing were between 14 and 18 days, showed varying degrees of cortical differentiation and duct development. They are not included in the discussion because the time elapsed at killing was under the minimum at which fully differentiated cortex had been observed, and, with but two exceptions noted below, none revealed development of cortex. One of these individuals injected on the 6th day, and 18 days old when killed, had a thickened epithelium with a suggestion of cortical cords in places. Another 16-day embryo, injected on the 4th day, appeared more like normal untreated embryos with a lumpy condition of the germinal epithelium.

In an attempt to determine just how late the germinal epithelium is modifiable by Progynon, embryos were injected as late as the 18th day of incubation. Embryos in this division of Group II, receiving 0.50 mg. Progynon, ranged in age from 10 to 18 days. Because of the late age at which the hormone was administered, a 14-day interval between treatment and killing did not elapse in all cases. A total of sixty-nine male embryos in this category was examined macroscopically, thirty-nine of which were sectioned and studied.

The gross structure of the gonads of these individuals was not greatly altered. In some instances there was a suggestion of flattening, which was not particularly striking. The margins varied from a smooth through a serrated condition to the previously noted small, finger-like projections. The right gonad was usually shorter, but not much so; and it was sometimes broader, as Figure 9 portrays. This same figure shows the rough corru-

gated surface on the left gonad alluded to before. This was observed in fourteen of the sixty-nine embryos injected from the 10th to the 18th day and killed from the 18th to the 28th day. It appears to result in large measure from primary sexual cords pressing against the surface and small lumps of persisting germinal epithelium.

There were numerous instances in which either a rather thick, almost continuous, outer layer or lumps more widely separated invested the sexual cords; but these structures were more similar in nature to the thickened outer layer described as persisting on the normal left testis than to differentiated cortical cords. One case was injected on the 10th day, and 10 days later, when 20 days old, it possessed a layer on the left gonad that was not unlike that found on the left gonad of a 10-day old untreated normal male embryo. One, injected on the 12th day and killed when 24 days old, showed a thickened layer that had the appearance of incipient cortical cords; but this was too doubtful to be recorded as certain. In a few cases the persisting patches of cells consisted principally of germ cells in groups of one to four or five. In eight cases all traces of the epithelium had been lost. This entire lot, injected from the 10th to the 18th day, can be said, therefore, to show no positive response, as far as cortical development is concerned.

With the Müllerian ducts a slightly different story is told. Stimulation here is not great but definite and in keeping with what might be expected from normal development, that is, persistence is greater at the anterior ends, where the final stage of degeneration of the Müllerian ducts in the male occurs. Among the sixty-nine cases in this lot, thirty-two showed remnants of ducts. In no speci-

men was the duct entire but, in the great majority of cases, consisted only of the anterior tips, as shown in Figure 9 (for summary, see Table 3). These results indicate that the ability to respond to hormone treatment is retained longer by the ducts than by the germinal epithelium covering the testes. Oviduct response was more pronounced in the female than in the male, as Figures 9 and 10 disclose. The two embryos illustrated were from the same lot of eggs, are the same age, and had the same treatment.

Group III.—These embryos received 0.75 mg. of Progynon. This was administered by injecting 0.25 mg. on the 4th day and 0.50 mg. on the 6th. The mortality rate was high, and from the thirty-two eggs originally injected, only four males and five females survived at the end of the 20th, 22d, and 26th days. No males were living on the 26th day, and the protocol states that, of the eight eggs removed at this time, three modified males and two females were dead and three females survived.

The two males killed on the 20th day revealed flattened gonads with irregular margins, and the left gonad of one showed protuberances on the surface. The two killed on the 22d day had rather slender, flattened gonads.

Gonads from three of the four males were studied histologically. The increased dosage exercised surprisingly little effect, for, at these ages, none showed a fully differentiated cortex. In all three cases young cortical cords were definitely in process of formation but had not attained the degree of development commensurate with the age and dose administered, since some of the cases described reveal better differentiation at 22 days with only 0.25 mg. Progynon injected on the 4th day. The difficulty here may rest with the small number of cases; yet the

fourth unsectioned case did not appear sufficiently modified to warrant sectioning.

The increase in dosage was readily apparent in the Müllerian ducts. One of the four males comprising this group had a left duct of normal length, greatly distended at the anterior end and hypertrophied at the posterior end. The right duct was large at its cranial end, as was the left, but it originated slightly anterior to the caudal end of the Wolffian body and extended to the cloaca. None of the other three had a complete duct on either side (see Table 3 for summary).

Group IV.—Dosage in this group of embryos was 1.00 mg. of Progynon administered on the 4th and 6th days in 0.5-mg. quantities. Nine individuals were studied macroscopically, and eight of these were sectioned. Age at killing ranged from 20 to 26 days. Grossly considered, the left gonad showed considerable evidence of posterior flattening in all cases; the flattening extended more anteriorly in five cases. In contour the margins of both gonads were irregular, flanked by detached lumps in some cases, and the surface of the left usually exhibited a lumpy condition. There was a disparity in size similar to the normal female in three instances, and in others one or both gonads were slender and elongated or not greatly altered.

The best cortical differentiation did not equal that of some embryos that received only 0.50 mg. Every individual exhibited a layer of variable thickness superimposed upon the testis cords, but its manner of origin appeared peculiar in some instances. Indications point to an outpushing of primary testis cords to the surface, followed by a multiplication of these on the surface (Fig. 33, Case 16P4622b, and Fig. 28, Case 16P4626c) which later become cut off to form a sec-

ond layer (Fig. 29). There are sections which show a single cord projecting alone and others that show this layer connected by tubules to those in the inner portion (Fig. 33). Perhaps such a method of formation requires a longer period and therefore accounts for the lack of fully differentiated cortex in this outer layer which was found in some treated embryos of a comparable age.

Only one case showed thickening that displayed differentiated cells, and here differentiation was incipient. Its cortical layer extended practically over the entire surface of the gonad. A transverse section showed the layer to be fairly thick on the medial surface but extremely thick at the margins of the hilus. There was a pronounced line of demarcation between the two layers, which became accentuated posteriorly, resulting in two layers of equal size, composed of cords of very different appearance (Fig. 29, Case 16P4626c). Although the outer layer was well developed, there was an absence of cells in synizesis at this level. Farther anteriorly, however, it showed a patch of fully differentiated cortex (Fig. 30). Figures 28, 29, and 30 are from the same embryo at different levels and apparently show testicular cords growing out, becoming cut off, and differentiated into cortex. As a rule, complete differentiation of the cortex begins anteriorly and proceeds posteriorly where it is thickest.

This group of embryos appears to furnish further proof that cortex may develop from young testicular tubules, as referred to earlier. A detailed study of all cases in our investigation seems to disclose two methods of cortical formation, namely, one from cortical cords formed from the germinal epithelium and the other from testicular cords, which grow out and become detached from the un-

derlying tubules. In the latter method a longer period is required for cells to appear in synizesis.

Müllerian duct development showed an improvement over Group III. Two of the nine in the lot showed development of the left duct throughout its length, and both of these displayed fluid-filled cystic enlargements at the posterior end of the right instead of the anterior. This latter condition is in keeping with normal development in the female (details of others are in Table 3).

Group V.—In this group the dosage was stepped up to 1.50 mg. This was given on the 4th, 6th, and 8th days in 0.50-mg. portions. All the embryos in this lot were killed on the 25th day, as experience had shown mortality on the 26th day, just prior to hatching, to be very high. Eight males survived for study, five of which were sectioned for histological details. Four of the eight showed a decided disparity in gonad size in favor of the left. In the other four, the left gonad was larger, too, but not so markedly. Considerable flattening was evident in the left gonad, as well as a lumpy surface, in all eight embryos of this group. The right was a bit more rounded in contour, but the margins were more irregular.

The microscope disclosed that the left gonads of three of the five embryos sectioned had pronounced cortical development. Among these, one had a very thick cortex, but most of its cells were in pre-synaptic stages, and there appeared to be a number of connecting cords between it and the primary sex cords. The other two cases showed cells in synizesis, hence were further along in differentiation. The remaining two of the five cases had a very thick layer of cells, containing some primordial germ cells, which was confined chiefly to the posterior half of the gonad

and did not extend across the breadth even here.

On the whole, Müllerian duct development was pronounced in this group. The left duct was either entire and quite large (four cases), large at the anterior end with intermittent cysts in the posterior half (two cases), or huge anteriorly with little or no hypertrophy at the posterior end (two cases) (see Table 3). The ducts of one of the latter were so large that they obscured the gonads and Wolffian bodies. All individuals of this group showed decided Müllerian duct enlargement. This, coupled with the fact that the best cortical development among the Progynon-treated birds was found in Group V, indicates that it is possible by increase in dosage to augment cortical differentiation. However, the augmentation with a dosage of 1.50 mg. over that following a dosage of 0.25 mg. did not appear to be commensurate with the increase in quantity. The Müllerian ducts were much more responsive to this stepped-up dosage.

2. *Females: Group VI.*—This group is comprised of the one hundred and seventy-five females treated with Progynon (0.25–1.50 mg.). While all these were examined macroscopically, only selected cases were studied microscopically. The principal effect of the hormone was registered upon the oviducts in the female, and a total of fifteen was selected for sectioning because they had either a large right rudiment, a very small right rudiment, or unusual Müllerian duct formations. These cases varied as to time of injection, age when killed, and dosage and are believed to give a good indication of the general effect of this hormone in the female.

The vast majority of the embryos reflected little or no change in ovarian structure as a consequence of Progynon

treatment. However, the most pronounced cortical development on the right ovary was observed in two individuals that received 0.50 mg. of Progynon on the 4th day and were killed on the 18th day. No female among the eleven normals studied histologically showed any cortical growth on the right gonad comparable to the condition found in these two cases. Indeed, one of these individuals showed development of cortex superior to that on the left ovary at the same level. It was a continuous layer plainly demarked from the underlying medulla and was as thick as this medulla. Macroscopically, the individual under consideration had shown a short, plump right gonad near the anterior end of the left, which ended abruptly, posteriorly. The other case was as thick as this in places but was not so fully differentiated, and the cortex occurred more in lumps than continuously.

Another female, which received 0.50 mg. of Progynon on the 4th day and was killed on the 25th, revealed the best development of cortex on the left found in all the embryos studied in this investigation. At about the mid-point in cross-section, the cortex of the left gonad at the lateral border of the hilus was enormous and appeared augmented by an extra lobe wedged in ventrolaterally. The ratio of the area of medulla to cortex in this section was approximately 1:5. The right rudiment in this embryo was unusually large and in cross-section displayed no well-defined cortex but a mass of lumps that appeared more like cortex than like medulla. Some of these lumps at various depths showed cells in synize-sis. One other female, which had received 0.25 mg. on the 4th day and had been killed on the 26th, showed cortical development similar to this case, but it was not quite so pronounced. The right

gonad here was unusually large also and displayed a better-defined cortical area than did the preceding individual.

These four cases suggest some cortical stimulation in females as a consequence of estrogen treatment but by no means prove it. We realize that they may simply be individual variations. In one paper, Dantchakoff (1935*b*) postulates that Progynon in females acts as a whiplash to speed up histogenetic differentiation with more vigor and rapidity. This may apply to some extent in the duck.

The Müllerian ducts of females appear to respond to Progynon in direct proportion to the dose and in inverse proportion to the age at the time of injection. Of twenty-seven cases aged 16 days and above, receiving 0.25 mg. at ages of 3–8 days, only six showed slight hypertrophy of the left duct and persistence of the right from the cloaca to the posterior level of the Wolffian body. Four others showed a posterior enlargement of the right duct at the cloaca. The general rule for the persistence of the right duct, as might be expected, is that hypertrophy occurs most often in the cloacal region and extends to higher levels in a decreasing number of instances.

As dosage is stepped up, there is an augmentation in diameter of the left duct and of length and diameter in the right with the younger injection ages (2–8 days). The highest dosage (1.50 mg.) produced the largest ducts, and both were entire in some females (three out of five), or both were much enlarged, with the right extending only about halfway (two out of five) (see Table 4).

Just how late the highest dosage of Progynon is capable of causing tumescence of the oviducts in females was not ascertained beyond question; but 0.50 mg. can cause a pronounced enlargement practically throughout the

length of the right and left ducts as late as the 12th day (Fig. 10). This amount was injected as late as the 18th day; and, of eight females, one showed slight hypertrophy of the left, and at the cloaca the right was somewhat enlarged; one showed slight enlargement in the left only; three revealed enlargement of the right posterior tip only; and three exhibited no hypertrophy of either. It may be noted in passing that, since it is about the 18th day that normal oviduct differentiation is complete, it is doubtful whether hormone administration following the 18th day would produce any noteworthy effect other than hypertrophy in the Müllerian ducts. A summary of the effects of Progynon upon the Müllerian ducts in females is given in Table 4.

B. RESULTS OBTAINED WITH STILBESTROL

Since Progynon failed to bring about complete sex reversal, it was decided to try other estrogens. Those utilized were diethylstilbestrol and hexestrol. These hormones are much more soluble in sesame oil, hence higher concentrations in the same quantity of fluid were possible. In the case of Progynon, $\frac{1}{20}$ cc. of solution contained only 0.25 mg. of hormone, whereas the same amount of sesame-oil solvent might contain 1.25 mg. or more of stilbestrol and hexestrol. Stilbestrol in the concentration used proved very toxic; hence only one lot of eggs was treated with this hormone. Table 5 summarizes some of the data obtained on the effects of stilbestrol.

1. *Males*.—Forty-five eggs were injected with 1.25 mg. of stilbestrol on the 4th day of incubation. The plan was to kill these embryos on the 26th day; but candling from time to time revealed so many dead embryos that on the 22d day, when only four remained alive, it was decided to kill these for fear that they

TABLE 4
EFFECTS OF PROGYNON ON MÜLLERIAN DUCTS OF FEMALES

No. OF EMBRYOS	DOSAGE (MG.)	INJECTION AGE (DAYS)	INCUBATION AGE (DAYS)	LEFT DUCT								RIGHT DUCT							
				Entire		¾*		½		¼		Entire		¾		½		¼	
				Hyp.†	Nor.‡	A.§	P.	A.	P.	A.	P.	Hyp.	Nor.	A.	P.	A.	P.	A.	P.
5.....	0.25	3	26		5														5
10.....		4	22-26		10														10
17.....		5	10-26	2	15								4		4		4		5
8.....		8	22-26	4	4									2		1			5
30¶.....	0.50	4	16-26	11	12	2	2	1			1		1		2		2	1	15
7.....		5	14-26	3	4								3		1		2		1
17.....		6	14-26	13	4							4	3		5		3		2
12.....		10	18-8**	12								2		8		2			
22.....		12	18-2**	21	1							1	1		4		6		10
17.....		14	20-8**	6	11														17
11.....		16	22-28	2	9														11
8.....		18	22-27	2	6														8
5.....	0.75	4 and 6	20-26	5										2					3
2.....	1.00	4 and 6	20-26	1					1					1					1
5.....	1.50	4, 6, and 8	25	5								3				2			

* The fractions indicate approximate lengths from either end.

† "Hyp." = Hypertrophied.

‡ "Nor." = Normal.

§ "A." = Anterior end.

|| "P." = Posterior end.

¶ One has no left Müllerian duct.

** Post-hatching age in days.

TABLE 5
DATA ON EFFECTS OF STILBESTROL

INJECTION AGE*	QUANTITY INJECTED (MG.)	No. OF EGGS INJECTED	EMBRYOS KILLED				SURVIVAL (PERCENT-AGE)
			Age*	No.	Male	Female	
4.....	1.25	45†	22	4	2	2	8

* Incubation days.

† The discrepancy between number of eggs injected and embryos killed is due to the death of the embryos in the interim.

would not survive until the 26th day. Examination revealed two males and two females.

One of the males displayed gonads with finger-like processes extending from the lateral margins, a condition similar to that observed when Progynon was administered. The left gonad was somewhat flattened and more ovary-like, while the right was more rounded. In the other male the gonads were very slender and elongate. The former male was chosen for sectioning because gonads similar to those of the latter, treated with Progynon, had been sectioned and showed scant cortical development.

A microscopic study of the gonads of the male disclosed a cortex on the left somewhat thinner than in normal females, with a few patches of cells in synzesis and flattened lumps of thickened epithelium at intervals on the right. The cortex on the left gonad covered most of its surface but, as usual, was thicker posteriorly and thinned out toward the anterior end.

Both males had huge ducts also; however, the enlargement did not extend all the way to the cloaca—only about two-thirds of the distance in the longer. Figure 12 shows the shorter of the two.

2. *Females*.—In the females, one right rudiment was large and one was small. The left ovaries showed nothing unusual in appearance. Figure 11 shows the female with the large right rudiment.

The ovary of the female with the larger rudiment, when sectioned, exhibited an unusually thick cortex, especially on the lateral border as seen in cross-section, which was reminiscent of the condition of the two Progynon-treated females discussed in Group VI. Most of the cells were in synaptic stages, except those on the median and the lateral borders. The right rudimentary gonad, although

larger than usual, did not possess a well-defined cortex but contained cortical and medullary cords not clearly demarked. Gonads of the other female were not sectioned.

The illustrations show that the stilbestrol-treated embryos noticeably surpassed the Progynon-treated ones in Müllerian duct development. Comparison of Figure 10 (best-developed Progynon case) with Figure 11 gives an idea of the difference. Both of these were drawn to the same scale and were 22 days old. The tumescence is more exaggerated in Figure 11. The second female showed ducts just about as large as this one.

C. RESULTS OBTAINED WITH HEXESTROL

Since hexestrol proved less toxic than stilbestrol, as indicated by a lower mortality, further observations were continued on the effects of hexestrol administration.

1. *Males Group I*.—For these embryos an attempt was made to put 25 mg. per cubic centimeter of hexestrol into solution in sesame oil. When this failed, the concentration was reduced to 12.5 mg. per cubic centimeter; but even with this dilution all the solute was not completely dissolved, and a fine suspension still pervaded the solution. This was used but was thoroughly agitated just prior to each injection. The quantity given was $\frac{1}{10}$ cc., which should have been equivalent to 1.25 mg., however, since the hormone was not completely in solution, there existed the possibility of variability of dosage. The number of surviving male embryos was five, two of which were killed on the 24th day and three on the 26th day.

The gonads of these five male embryos bore evidence of strong hormonal influence. In all cases there was considerable difference in size in favor of the left. Both

gonads had very irregular lateral margins and were somewhat flattened, especially the left, which also was broader in most of the cases. The urinogenital system of one of these interesting cases is shown in Figure 13. This illustrates about as great a disparity in size of gonads as was obtained.

The most complete cases of reversal in this study, as shown histologically, were found in two individuals of this group. One, killed on the 26th day (illustrated in Fig. 13), possessed a cortex on the left testis as fully differentiated and

as thick as the untreated female (Fig. 25). This differentiated cortex extended from about the middle of the gonad to the posterior end. The underlying tissue was definitely testicular in character, as the illustration shows. Figure 26 shows an enlarged section of this cortex with cells in synizesis similar to those of the normal female, shown magnified more highly in Figure 17. From the mid-point anteriorly, the cortex became thinner, terminating in a scant layer at the anterior end of the gonad. The cortex on the caudal half was set off from the under-

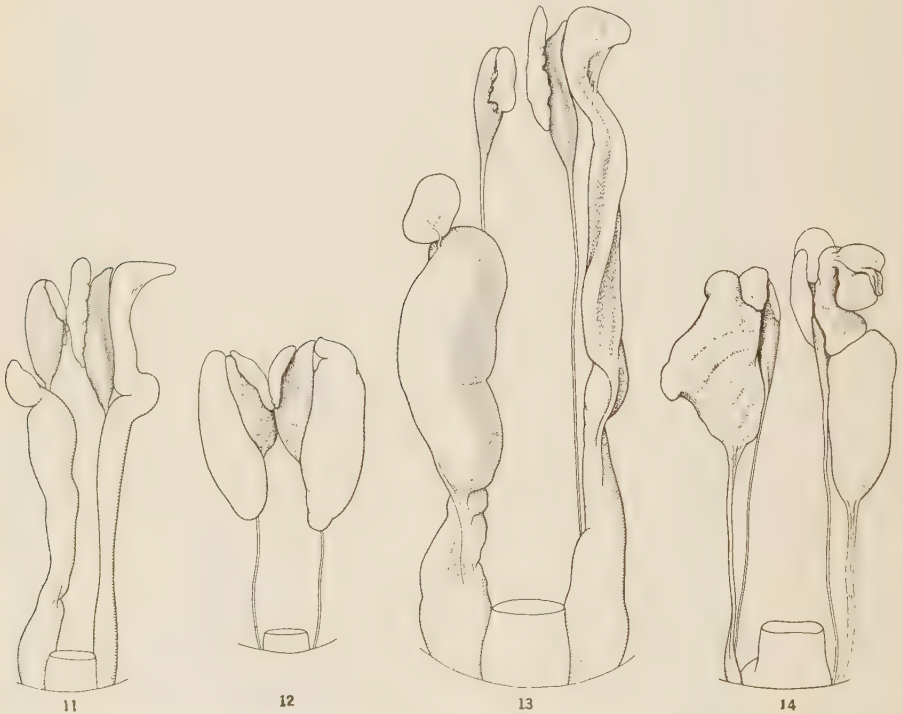


FIG. 11.—Reproductive system of genetic female embryo treated with 1.25 mg. stilbestrol on 4th day. Age 22 days. Note large right rudiment and compare duct development with Fig. 10.

FIG. 12.—Reproductive system of genetic male embryo of same age and treatment as Fig. 11. Compare duct development with Fig. 9.

FIG. 13.—Reproductive system of genetic male embryo injected with 1.25 mg. hexestrol on 4th day. Age 26 days. Note disparity in gonad size and enormous oviduct development. This represents the most highly modified condition obtained in males.

FIG. 14.—Reproductive system of genetic female embryo of same age and treatment as Fig. 13. Observe the enormous development of the anterior ends of the oviducts to the exclusion of the posterior.

lying tubules by connective tissue. There was no evidence of medullary cords of the female type, those underneath the cortical layer being clearly recognizable as testicular in nature. The entire urogenital system of this individual is shown in Figure 13.

The surface of the right gonad showed fairly thick lumps of epithelium in places. Very remarkable, however, was the appearance of a layer of cells, some approaching synizesis, on the median dorsal surface of the right Wolffian body, some distance posterior to the terminal portion of the right gonad. It was definitely cortical in appearance.

The other male embryo which displayed a high degree of reversal belonged to the same lot as the one just described, had the same dosage, and was killed at the same age. Macroscopically, the right and left gonads also showed about as great a disparity in size. Although the cortex on the left gonad was practically as well developed, the outstanding difference was the relationship existing between the cortex and the rest of the gonad. The former gave the appearance of something superficially imposed or tacked onto the medulla, with considerable connective tissue between (see Fig. 25). This relationship did become somewhat more intimate toward the cranial end of the gonad, whereas, in the latter individual, the cortex appeared to have the same relationship to the underlying portion as the normal female cortex has to the underlying medulla (Fig. 27). This underlying area, however, was not typically female. Two types of tissue were distinguished, namely, connective tissue composed of elongated, flattened cells, and testicular tubules.

The three remaining male embryos in this group showed lesser grades of gonad transformation. The 26-day male, which

had unusually long, thin, slender gonads, with the left much longer than the right, exhibited only a thin layer of cortex on the left gonad in cross-section. The layer was a bit thicker at the caudal end but not unusually so. The other two were killed on the 24th day. One displayed good cortical development, but it was not on a par with the best. The second 24-day embryo possessed cortex of only medium thickness on the left gonad, with fairly numerous groups of cells in synizesis.

Müllerian duct hypertrophy was so enormous that it approached the bizarre (Fig. 13). The ducts were huge in all cases and were either entire on both sides (three cases), entire on the left and almost so on the right (one case), or ballooned only at the cranial end on both sides (one case). In one of these birds the ducts obscured the gonads entirely, making dissection of them without injury extremely difficult. Duct development following hexestrol administration surpassed by far even that produced by stilbestrol (see Table 7).

Group II.—This group will be considered in two lots—those injected on the 4th, 6th, or 8th day to determine to what extent the germinal epithelium may be modified by heavy dosage of hexestrol and those injected at from 9 to 18 days to ascertain the period at which induction of cortex is no longer possible. The dosage for this group was 2.50 mg.

The first lot consisted of twenty-seven male embryos, which were killed from the 19th through the 26th day. It was originally intended that they should be taken on the 26th day, but the high rate of mortality made it necessary to change to the 25th day or earlier. Table 6 shows the survival percentage to be exceedingly low.

Gross study showed that all ten of the

male embryos injected on the 4th day possessed gonads dissimilar in size, a condition which was obvious at a glance. It was the left gonad that was larger, and very decidedly so in four cases. Often the gonads were so crowded because of tumescence of Müllerian ducts that the shape was not readily apparent; but, in

one end of the left gonad to the other, and one showed cells in synizesis at the posterior tip, which has been noted only in a high-grade intersexual type. Two had cortex equivalent to the poorest developed in normal females of the same age, and these gonads might readily have been taken for ovaries, had it not been

TABLE 6
DATA ON EFFECTS OF HEXESTROL

INJECTION AGE*	QUANTITY INJECTED (Mg.)	NO. OF EGGS IN- JECTED	EMBRYOS KILLED				SURVIVAL (PERCENT- AGE)
			Age*	No.	Male	Female	
4.....	1.25	45	24-26	21	5	4	20
4.....	2.50	145	25	33	6	2	11
6.....		45	19-25	27	9	5	31
8.....		30	19-25	17	8	6	46
9.....		25	23-27	12	8	3	44
10.....		29	25-28	17	4	1	17
12.....		53	22-2†	35	12	13	36
14.....		62†	25-4†	44	11	7	29
16.....		30	28-2†	8	5	3	26
18.....		16	27-4†	15	2	2	25
4 and 6.....	3.75	60					(All dead by 10th day)
Totals of sexes.....					52	37	
Sex ratio (per cent)					58	42	

* Incubation days.

† Post-hatching.

‡ The discrepancy between number of eggs injected and embryos killed in all cases in the table is due to the death of embryos in the interim.

general, the left was flatter, and the right, which had a regular lateral margin, was only slightly more rounded.

The gonads of nine of the ten male embryos were sectioned and studied further for histological changes. In five embryos the left gonads showed transformation of the same general quality and were decidedly of a high-grade intersexual type but somewhat inferior to the two described in Group I, with regard to amount of cortex. In all five cases there was good development of cortex from

for the unmistakable male character of the underlying tubules. The right testis in none of these individuals developed a definite cortical layer.

The other four cases showed varying lesser degrees of cortical thickness on the left gonad, but all exhibited at least a few fully differentiated cells. Reference to Table 7 will disclose that the dosage employed was instrumental in producing cortex that showed cells in synizesis in every treated male in Group I and those injected on the 4th day in Group II. De-

spite this fully differentiated cortex on the left testis in all cases, there was no transformation of testicular cords into medullary cords—hence no complete inversion. No definite cortical layer was found on the right testis.

The other individuals comprising lot No. 1 of Group II were those injected on the 6th day (nine cases) or the 8th day

Hypertrophy of Müllerian ducts in this lot was exceedingly great also. Six of the ten injected on the 4th day had enormous, full-length oviducts on both sides (see Table 7 for greater details). These ducts were so large that they often covered the gonads entirely and, coupled with the viscera, sometimes pressed them into abnormal shapes.

TABLE 7

SUMMARY OF THE EFFECTS OF HEXESTROL ON GONADS AND MÜLLERIAN DUCTS OF MALES

No. of Embryos	Dosage (Mg.)	Injection Age (Days)	Incubation Age (Days)	GONADS			MÜLLERIAN DUCTS														
				Differentiated Cortex*	Undifferentiated Cortex	No Definite Cortex	Left Entire	Left Inc.†						Right Entire	Right Inc.						No Ducts
								2†		1‡		1			2		1‡		1		
								A.§	P.¶	A.	P.	A.	P.		A.	P.	A.	P.	A.	P.	
5...	1.25	4	24-26	5			4	I					4	I							
10...	2.50	4	25-26	10			7	I		2			6		I	3					
9.....		6	19-25	2	5	2¶	2	4		3			5		3	I					
8.....		8	19-25	2	4	2		I		2		5			I		7				
8.....		9	23-27	2	I	5		I		3		4			2		6				
4.....		10	25-27		I	3		I		I		2			I		I				
12.....		12	22-2**			12¶				4		8			I		3		8		
11.....		14	25-4**			11¶						6					6		5		
5.....		16	28-2**			5¶				2		2					2		3		
2.....		18	27-4**			2¶						I					I		I		

*"Differentiated Cortex" refers to cortex with cells in synyzisis and they may be few or many. "Undifferentiated Cortex" indicates cortex lacking cells in synyzisis.

†"Inc." = Incomplete.

‡ The fractions indicate approximate lengths from either end.

§ "P." = Posterior end.

|| "A." = Anterior end.

¶ Not all sectioned.

** Days post-hatching.

(eight cases). Two of the males treated on the 6th day showed fairly thick, fully differentiated cortex only slightly inferior to those injected on the 4th day, and five other males displayed undifferentiated cortex on the left gonad. Among those treated on the 8th day, fully differentiated cortex on the left gonad, not quite so good as that shown by those injected on the 6th day, was found in two individuals and undifferentiated cortex in four (see Table 7 for ages, etc.).

Müllerian duct development in embryos injected on the 6th or 8th day was not quite so good as that of those injected on the 4th day, as comparison in Table 7 will show.

The second lot in this group received a dosage of 2.50 mg. of hexestrol either on the 9th, 10th, 12th, 14th, 16th, or 18th day. A total of forty-two males was studied (see Table 7 for distribution and age when killed).

Macroscopically, the testes did not

show any striking deviation from the normal. There were three cases among the 9-day injected embryos in which the left testis appeared a bit larger than the right, more so than in untreated birds, where there is usually a slight disparity in size in favor of the left. Also, one 10-day treated and one 14-day treated embryo exhibited this abnormal size difference. The surface of the gonads varied from a more or less smooth surface through fine projections to coarser lumps, which never attained the broad proportions characterizing good cortical development. There was slight flattening of the left in a few instances, but for the most part it was typically rounded.

Of this lot, thirty-four were sectioned for evidence of cortical differentiation on testes. Three of the embryos injected on the 9th day and killed on the 24th or 27th day showed cortex recognizable as such on the left testis but not especially well developed. Two of these showed some cells in synizesis. It was in these that the left gonad showed up definitely larger than the right and that the lumps on the surface were largest. Those that possessed the finer elevations showed primary sex cords that grew out to the surface in places and sometimes protruded into the coelom. These protrusions appear to account for the finer surface elevations.

Only one of the four embryos injected on the 10th day displayed what appeared to be cortex on the left testis. Its left gonad before sectioning was characterized by the larger-sized lumps. This cortical development was poor in contrast to that of embryos injected with a similar concentration at an earlier age, 4-8 days, with an equal time interval elapsing between injection and killing.

Since Müllerian duct degeneration is not complete before the 18th day, it was

decided to extend the injections to that day in order to determine how late they could be modified by hormone administration, as well as to determine when induction of cortex stops. After the 10th day it appears that cortex is no longer developed from the germinal epithelium. This conclusion is based on the results of the study of thirty embryos injected from the 12th to the 18th day of incubation (see Table 7). Some of these embryos, however, showed an outgrowth of testicular tubules in the left gonad to the surface, where they became cut off to form lumps resembling cortex. Figure 34 illustrates one of the best-developed cases and is taken from a 28-day embryo injected on the 14th day. Whether such lumps would have differentiated into cortex if allowed to continue to develop was not determined. It appears significant, however, that these tubules should develop most frequently and to a greater degree on the left testis and should appear only occasionally on the right, where, when found, they are poorly developed.

It appears that, as Müllerian duct regression proceeds, estrogens are powerless to cause development of these ducts in the caudal segment, where regression has begun. Therefore, varying lengths of oviducts will develop when estrogens are injected, depending upon the age at which the hormones are administered. As a general rule, allowing for individual variations, the later the injection after the 4th day, the shorter the anterior persisting segment. Or, in other words, the older the male embryo at treatment, the greater the distance between the cloaca and the persisting portion of the Müllerian duct. This idea is brought out in Figure 15, where the lengths of oviducts of embryos of from 4 to 18 days' incubation are represented. A complete sum-

mary of the effect of hexestrol upon the Müllerian ducts of males is found in Table 7.

Group III.—Failure to achieve complete inversion of male gonads with the dosage employed in Group II led to the attempt to accomplish this end with higher dosages of hexestrol. Accordingly, 3.75 mg. of hexestrol were injected in each of sixty eggs. On the 4th day 1.25 mg. was administered, followed by 2.50 on the 6th day. Candling showed that the embryos succumbed very rapidly. By 2 days after the second injection, all were dead but one, and this one died on the 4th day.

2. *Females: Group IV.*—This group contains all the hexestrol-treated females, totaling forty-nine in number. In general, the estrogens did not appear to exert any marked effect upon the gonads, but some unusual conditions of both Müllerian ducts and ovaries may be recorded.

The abnormal gonad structure manifested itself as a very thin left ovary, with decided finger-like projections extending from the lateral and caudal margins. This abnormality in some cases was limited to the posterior half of the ovary. Seven of the forty-nine, or one-seventh of the number, showed this trait.

Sectioning and study of these unusual ovaries revealed interesting situations. The case illustrated in Figure 14 and another embryo of the same age and treatment, dosage 2.50 mg. hexestrol, which gave the same general macroscopic picture, were shown microscopically to have atypical left ovaries. These exhibited a cortical region composed largely of cells with flattened nuclei, and only a few cells with typically rounded nuclei were occasionally interspersed. Dantchakoff (1936) labels similar-appearing cortex in the chick, where all the nuclei are flat-

tened, as "sterile." The Progynon-treated female, dosage 0.50 mg., with missing left Müllerian duct, mentioned below (right has small posterior cyst), showed a high degree of "sterility" also. Strangely enough, the female among these Progynon-treated birds that had the best cortex on the right rudiment had a left ovary largely "sterile" and the anterior end of the left oviduct missing. The best-developed cortex on the right rudiment seen anywhere (Case 21H1425b) was treated with hexestrol (2.50 mg.), and in this instance the posterior end of the left oviduct was missing. The right was entire. The left ovary in this case was not "sterile" but smaller than usual, with good cortical development. The right ovary was as well organized into cortex and medulla as any left ovary, and the cortex was as thick as the average, with many cells in synizesis. In other words, it was a typical ovary but of small size. Another hexestrol-treated (2.50 mg.) female, which had a narrow right ovary, mostly cortex of normal thickness, had the posterior end of the left oviduct absent, no right duct, and a left ovary largely "sterile." A final case with deficiency of the left Müllerian duct at the anterior end (right had small posterior cyst) was treated with Progynon (0.50 mg.). Here the right cortex was of normal thickness, and the left, although not "sterile," was retarded in development.

In hexestrol-treated embryos the left Müllerian duct may (1) be totally absent, (2) have varying lengths of its anterior half absent, or (3) have varying lengths of its posterior half missing. The right duct showed these variations, too; this was not so surprising, since it does not persist in its entirety in the untreated female but normally disappears in an anteroposterior direction and seldom, if ever, shows a well-developed cranial

end to the exclusion of the caudal. Figure 14 pictures one of these unusual cases of duct development in the female, which is much more like the male condition treated at a later age. A few similar instances were noted with the higher dosages of Progynon. A summary of the effects of hexestrol upon the Müllerian ducts of females is given in Table 8.

It was stated previously that the right rudimentary ovary in female duck embryos is rather large, as compared with the right rudiment in the chick. Twenty-one estrogen-treated females were sectioned, and thirteen of these showed cor-

tical development (approximately 62 per cent). Some of these displayed only a few lumps, but others had a thick and well-differentiated cortex comparable to the normal, as mentioned above. It must be remembered, however, that these cases were not picked at random but because of an unusually large or small rudiment or some unusual feature of duct development.

D. RESULTS OBTAINED WITH TESTOSTERONE PROPIONATE

The only androgen utilized in this investigation was testosterone propionate.

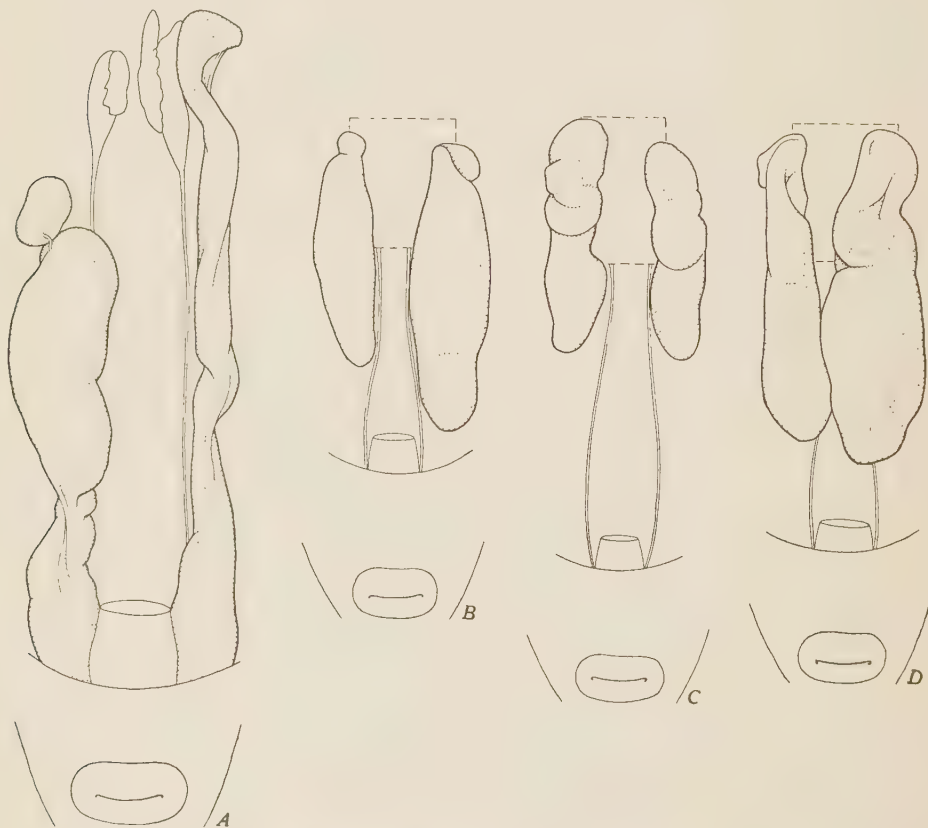


FIG. 15.—Illustrations showing persisting segments of oviducts in males treated with hexestrol, length determined by age of embryo at time of injection. The anterior end is usually the last portion to persist; hence the younger the embryo at time of injection, the longer the segment. Dosage in each instance was 2.50 mg. except *A*, which received 1.25 mg. *A*, injected on 4th and killed on 26th day post-hatch; *B*, injected on 6th and killed on 19th day of incubation; *C* and *D*, injected on 9th and killed on 24th day.

Data on its effects are summarized in Table 9. The dosage was 1.25 mg., and it was administered on the 5th day. The embryos were killed from the 18th to the 26th day. Survival was approximately 30 per cent.

No great deviation from the normal was observed in the eleven surviving females and eight males. The gonads appeared to be normal macroscopically, with possibly one exception in one female, where the cortex was sloughed off in places. In neither sex was there any apparent effect macroscopically upon the Wolffian or the Müllerian ducts.

The gonads of three males and three females were sectioned and examined histologically. Other than the sloughing-off of some cortex in the female (case previously mentioned), no unusual features were observed. Because there were no indications macroscopically of stimulation in the others and since the results obtained from those sectioned were negative, no more embryos were prepared for histological study.

E. RESULTS OF HISTOLOGICAL STUDY OF SEXUAL DUCTS

It has already been mentioned that Müllerian duct hypertrophy was greater

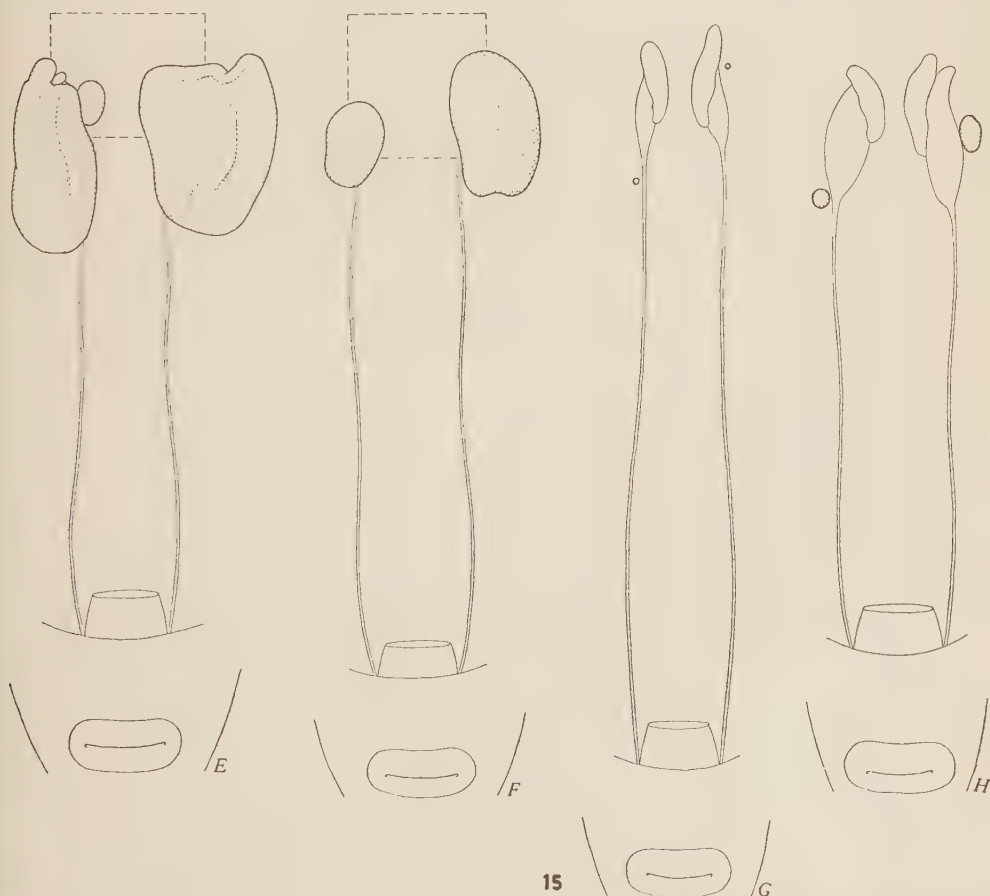


FIG. 15 (cont.)—E, injected on 12th and killed on 26th day; F, injected on 14th and killed on 25th day; G, injected on 16th and killed on 2d day post-hatch; H, injected on 18th and killed on 27th day of incubation.

in the embryos treated with hexestrol than in those treated with Progynon. In addition to the difference in size, there was a difference in the general macroscopic appearance, which seemed to be occasioned largely by the difference in thickness of their walls. The embryos treated with Progynon possessed ducts that were usually thin-walled, fluid-filled, more or less rounded, and opales-

1. *Müllerian ducts in normal females.*—Sections made from a segment of the left Müllerian duct just posterior to the Wolffian body from 26-day females reveal a tube rather oval in outline. The contour of the lumen, however, is not smooth and oval but presents a scalloped effect. The lumen is lined with a layer of columnar epithelial cells, which rest upon a layer of connective tissue that is, in

TABLE 8
EFFECTS OF HEXESTROL ON MÜLLERIAN DUCTS OF FEMALES

NO. OF EMBRYOS	DOSAGE (MG.)	INJECTION AGE (DAYS)	INCUBATION AGE (DAYS)	LEFT DUCT						RIGHT DUCT						
				Entire		$\frac{3}{4}$ *		$\frac{1}{2}$		Entire	$\frac{3}{4}$		$\frac{1}{2}$		$\frac{1}{4}$	
				Hyp.†	Nor.‡	A.§	P.	A.	P.		A.	P.	A.	P.	A.	P.
4.....	1.25	4	24-26	3				I		3			I			
7.....	2.50	4	25-26	3		I	I	2		5						2
5.....		6	19-25	5						5						
6.....		8	25	6						6						
2.....		9	24-25	2						2						
4.....		10	25-28	4						4						
9.....		12	22-2¶	9						7		I		I		
7.....		14	25-4¶	7										7		
3.....		16	2¶	3										2		I
2.....		18	4¶		2											2

* The fractions indicate approximate lengths from either end.
† "Hyp." = Hypertrophied.
‡ "Nor." = Normal.
§ "A." = Anterior end.
|| "P." = Posterior end.
¶ Post-hatching age in days.

cent in color. Because of the thinness of the walls, puncture caused almost complete collapse after the aqueous fluid escaped. The hexestrol-treated embryos had ducts that were thick-walled, filled with a more viscid fluid, somewhat flattened and ruffled, and without opalescent lustre. Escape of fluid caused little alteration of the shape. The estrogens employed had no effect on the Wolffian ducts of either sex, nor did we observe any modifications in Müllerian ducts as a consequence of androgen treatment.

turn, surrounded by a thin muscle layer. The vestigial right oviduct of the normal female was not sectioned.
2. *Müllerian ducts in estrogenized males.*—Histologically, the lower dosages that result in the persistence of the oviducts in the male may produce a condition which is essentially similar to that described for the normal Müllerian duct in the female. As the dosage is increased, distention of the oviduct beyond the normal size because of collected fluids results in a thinning of the oviductal wall, so

that there is a smooth epithelial lining minus folds, inside a very thin muscle layer. In cross-section the lumen was oval shaped and regular in outline, while the lining epithelium was reduced from the tall columnar type to the flat or cuboidal condition, probably because of the pressure of the inclosed fluid. The highest dosages of Progynon produced in one or two instances a thicker wall, occasioned by the evagination of the epithelium into glandlike diverticula, complicating the connective tissue of the wall. A thin muscle layer was added to the outside. The right oviduct in treated males

along the margin of the surface bordering the lumen. The contour of the lumen, therefore, was very irregular in outline, as compared with most of those treated with Progynon.

Some males, treated late in the incubation period, developed small globular cysts in the vicinity of the anterior end of the degenerated Müllerian duct. Such were assumed to be persisting portions of these ducts, and sectioning confirmed this assumption.

3. *Müllerian ducts in estrogenized females*.—Only the higher dosages of estrogens caused the persistence of the right

TABLE 9
DATA ON EFFECTS OF TESTOSTERONE

INJECTION AGE* (DAYS)	QUANTITY INJECTED (MG.)	NO. OF EGGS INJECTED	EMBRYOS KILLED				SURVIVAL (PER- CENTAGE)
			Age*	No.	Male	Female	
5.....	1.25	65†	18-26	49	8	11	0.29
Sex ratio (per cent).					43	57	

* Incubation days.

† The discrepancy between number of eggs injected and embryos killed is due to the death of the embryos in the interim.

showed all the histological features found in the left and, in addition, was found degenerated into a short vestige, as may be found on the right in the normal female.

A cross-section from the same region as in females shows how tremendously the glandular layer has been augmented following hexestrol treatment (2.50 mg.), as compared with Progynon treatment (1.50 mg.). The walls become exceedingly thick and exceedingly glandular, causing the muscular layer to become reduced in places from a compact layer to a thin, mere network of muscle cells. The columnar epithelium lining the lumen dips deeply toward the muscle layer at intervals, producing an interruption in the continuity of the thick-walled condition

oviduct in its entirety, and for that reason it was found with less frequency among the embryos treated with Progynon than among those receiving hexestrol (see Tables 4 and 5). Histologically, the right duct behaves about the same as the left does in response to varying dosages, and both right and left ducts conform to the description given for estrogenized males.

4. *Wolfian ducts in normal males*.—In view of the marked effect of testosterone upon the mesonephric system of the chick (Dantchakoff, 1937; Willier, Rawles, and Koch, 1938) and the apparent lack of effect in the duck with the amount injected, as determined macroscopically, it seemed desirable to make histological

preparations of the Wolffian ducts. Segments about the length of the gonads were taken just posterior to the Wolffian body and almost immediately anterior to the cloaca and were sectioned. When microscopic examination disclosed no readily observable difference between these sections and those of control embryos, ocular micrometer measurements were made of the lumen and the entire diameter of the Wolffian ducts. Three readings were made from the slide containing serial sections taken from the anterior segment of the duct, and three from the posterior segment. The sections chosen for measurement were from approximately the beginning, the middle, and the end of the slide. These figures were averaged for all the individuals in a particular category and were compared with the averages of controls obtained likewise.

The sections from the anterior end of the duct in the normal individual generally exhibited a lumen more oval than circular; it was lined by a layer of columnar epithelial cells slightly larger than the lumen, with the result that there was some buckling of the epithelial layer into the lumen. Outside the epithelium was a thick wall of connective tissue surrounded by a thinner muscular layer.

Sections from the posterior segment were decidedly more oval in contour, indicating compression. There was a considerable increase in the entire diameter of ducts; the average through the long axis was three times that at the anterior end. The diameter of the lumen at the posterior end was almost four times the anterior lumen diameter. At the posterior level the lumen was very irregular because of infolding of the mucosal lining, and the increase in diameter of the duct was due to the larger lumen, as well as to augmentation in thickness of the muscular wall.

5. *Wolffian ducts in androgenized males.*—Sections of Wolffian ducts were obtained from the anterior and posterior ends in the manner described for normal males and were examined microscopically. Ocular micrometer measurements of the diameter of the lumen and of the entire duct were made. The average reading at the anterior level was a fraction greater in the treated than in the untreated, but at the posterior end the fractional difference was in favor of the normal. These differences tend to support the observations made macroscopically that there appears to be no definite effect produced by testosterone upon the Wolffian ducts under the conditions given.

6. *Wolffian ducts in androgenized females.*—Sections made from the anterior and posterior ends of the Wolffian ducts did not show any indication of stimulation. Measurements of the diameter of the entire duct and of its lumen were made at the two levels above mentioned in both normal and androgenized females, and the readings showed a greater average diameter of the ducts in the untreated females at both ends.

From these data it appears that, on the whole, there has been little or no hypertrophy of Wolffian ducts as a consequence of testosterone treatment with the relatively high dosage administered.

V. DISCUSSION

Detailed investigations of the origin of the germ cells and the structure and development of the sex glands in the chick have been carried out by a number of investigators, notably Swift (1914, 1915, 1916), Firket (1914), D'Hollander (1904), Semon (1887), and Hoffman (1892); but only Laulanié (1886) mentions a homologue of the ovarian cortex, which he refers to as a *couche ovigène* that may be observed on the left testis be-

tween the 7th and the 12th days of incubation. In referring to this work, Swift (1916) states: "According to his idea, the gonad was not indifferent but hermaphroditic. Later on in development one of the sexual elements degenerated and the gland became male or female as the case might be. This idea is of interest only because of its novelty and historic interest." Swift's detailed description of the embryonic development of the testes fails to include any mention of a persisting thickened germinal epithelium on the left testis. Concerning the epithelium, he states that by the end of the 9th day of incubation the germinal epithelium is reduced to a single layer of cuboidal cells.

Since these observations were made by Swift, it has been demonstrated that Laulanié was correct in postulating a bisexual condition obtaining in the embryonic left testis of the chick (Dantchakoff, 1936; Willier *et al.*, 1935, 1936, 1937; Wolff and Ginglinger, 1936a); and representatives from the other two classes of Amniotes—Reptilia and Mammalia—as well as additional Aves, have been shown to possess bisexual gonads bilaterally or sinistrally. Among reptiles, Risley (1933) demonstrated bisexuality in the musk turtle, Forbes (1938) in the alligator, Dantchakoff (1938) in the lizard; among birds other than the chick, Burwell (1931) in the duck, Witschi (1935) in the sparrow; and among mammals, Hett (1932) in the cat, dog, squirrel, mouse, rat, guinea pig, sheep, calf, pig, and man.

In normal development the bisexual components of the testis experience various fates. According to Risley (1933): "Both testes and ovaries develop out of a bisexual primordium, and the bisexuality of the early gonads furnishes a morphological foundation for the eventual formation of either sex." In the male turtle

the distinct ovarian cortex which is formed during embryonic development gradually retrogresses. Witschi (1935) reports a similar condition to that in the musk turtle as obtaining in the common sparrow. The male chick possesses this potential cortex in the form of a thickened persisting germinal epithelium "from at least the seventh to the eleventh day," after which it undergoes retrogression (Willier, Gallagher, and Koch, 1937).

Grünwald (1934a, b) gives an account of the normal development of the sexual cords in several mammals, namely, the rat, mouse, pig, and man; and in the latter he describes fully the development of structures which he designates as secondary sexual cords that are analogous to the cords of second proliferation in the female; therefore, he calls them the "secondary sexual cords of the male." He states that no one had given an account of the development of these cords in the male, as far as he was able to ascertain in his survey of the literature. He mentions Hett's (1932) observation of a persisting germinal epithelium in certain mammals, but Hett did not record the development of this epithelium into the secondary sexual cords.

According to Grünwald's (1934b) account, there develops on both testes of the human embryo, outside or within the tunica albuginea, sexual cords that differ from the primary cords by the size and staining affinities of the cells. As these cords develop, the tunica, which separates the primary and secondary cords, disappears; and a new tunica is formed outside the secondary cords, resulting in the intimate contact of the two sets of sexual cords. Not only do these cords have contiguity, but union is established between them, and within a short period it is impossible to distinguish primary

from secondary cords. These secondary cords are regarded as homologous to the cords of second proliferation in the female, but they do not arise at a fixed time as in the female, neither were they found in all the embryos examined. At the time, Grünwald was not sure whether they were present in all embryos or whether they had escaped his notice because he did not examine the gonads at the propitious moment for their observation, since he states that their time of development is not predictable as in the ovary and that they, soon after their formation, blend indistinguishably with the primary sex cords and all become incorporated within the same tunica. Hence he concludes that fundamentally there is no difference between the human ovary and testis with reference to the origin of the sexual cords. The actual difference is in the manner of participation of the two sets of cords in the formation of the gonads as a whole; there are fewer secondary cords involved in the architecture of the testis than in that of the ovary.

The observations in our investigation upon the normal development of the left testis of the duck appear to have much in common with those of Grünwald upon the human testis. In the duck, which follows the asymmetrical gonad development found in most birds (Domm, 1927), the formation of secondary sexual cords is limited chiefly to the left testis, with only an occasional feeble attempt on the right; whereas, in man, secondary sexual cords are found in both gonads (Grünwald, 1934*b*). These secondary cords in the duck increase in size by proliferation and division of germ cells, located in the persisting germinal epithelium found on the left testis (Burwell, 1931), so that this layer becomes characterized by lobu-

lations, as observed for cortical-cord formation in the chick (D'Hollander, 1904; Firket, 1914; Swift, 1915); however, these lumps of cells in the duck may cause surface elevations, as well as protrusions inwardly, which encroach upon the tunica albuginea. Continued proliferation of cells in these lobulations results in the production of definite cords, which appear analogous to the secondary sexual cords shown in Grünwald's (1934*b*) illustrations of the human embryonic testis. These cords in the duck also are readily distinguishable from the primary cords by a difference in affinity for certain stains. They form a conspicuous investing outer layer on the gonad. The layer is always thicker at the posterior end of the gonad and in some cases is lacking entirely at the anterior end. The final disposition of this layer of cords was not determined with certainty; however, there is some evidence that they may be incorporated in the testicular structure and become indistinguishable from the cords of first proliferation, as postulated by Grünwald for the human embryo.

Studies on the structural composition of the ovary of birds, as well as of mammals and certain other vertebrates, have clearly shown a bisexual organization. The primary sex cords which constitute the medulla are morphologically equivalent to the sex cords of the testis, while the cortex formed from the secondary sex cords is distinctly ovarian. Analysis of the freemartin condition (Lillie, 1917) demonstrated that the medulla is not only the morphological homologue of the testis but is also potentially a testis. A similar condition prevails in birds, where the right rudimentary gonad, composed chiefly of medullary tissue, develops into a testis, following sinistral ovariectomy (Benoit, 1923, 1924, 1926; Domm, 1924,

1927, 1929, 1931; and others). In each case the first set of sexual cords differentiates into seminiferous tubules.

The bisexual organization of the ovary is not peculiar to birds and mammals but appears to be demonstrated also for Amphibia, as shown by the studies of Witschi (1929, 1934), Burns (1935), and Humphrey (1935), as well as for reptiles, as revealed by the observations of Risley (1933) in the musk turtle and Forbes (1938) in the alligator. It is therefore clearly demonstrated that the ovary of many, if not all, vertebrates consists morphologically of a rudimentary testis, the medulla, surrounded by the specifically female cortex, and that, as has been equally well shown, this morphological composition is an expression of corresponding physiological capacities. Hence the potentialities for sex inversion in the female were demonstrated long before this condition was known in the male.

The first attempts to duplicate the freemartin condition were undertaken in the form of grafting experiments. Minoura (1921), who pioneered in this work on the chick, claimed to have obtained sex reversal by gonad transplants; but Greenwood (1925), Kemp (1927), and Willier and Yuh (1928) did not agree with the findings of Minoura. Willier and Yuh demonstrated that gonad grafts were nonspecific in producing sex reversal and that results similar to Minoura's could be induced by certain conditions of incubation, such as low temperature, and by various operative procedures, as well as by grafts of nongonad tissue. So the expedient of grafting, which was successful in bringing about sex reversal in Amphibia (Witschi, 1927; Humphrey, 1929; Burns, 1931), was a failure in so far as the chick was concerned.

With the isolation and purification of sex hormones and their ready availability in pure form, this problem was reinvestigated with more fruitful results. Kozelka and Gallagher (1934) injected estrogens into hen's eggs; and, following their success in inducing the left gonad of the male to develop into an ovotestis, subsequent investigators have demonstrated complete transformation of the left testis into an ovary in the chick embryo. Willier *et al.* (1937), using sex-linked chicks, observed that theelin and theelol, in dosages of 1.0 and 2.0 mg. injected into the albumen, caused complete inversion of the left testis into an ovary and the right testis to become reduced in size and flattened and to develop ovarian medullary tissue, though its testicular character was never completely lost. Wolff and Ginglinger (1935a) found that approximately 100 I.U. of folliculine, when applied directly to the blastodisk, would produce an intersexual condition in all male embryos and that 500 I.U. or over were required to bring about complete reversal. Dantchakoff (1936), by the introduction of 2,500 mouse units of folliculine into the allantoic sac, stated that she was able to bring about complete sex reversal in the male chick at will.

In the present investigation no case of complete reversal of the left testis of the duck was observed, despite the fact that dosage was stepped up to a point (3.75 mg. of hexestrol) at which all embryos of sixty eggs injected were dead by the 10th day of incubation. The initial dosage was 0.25 mg. of Progynon, and this was gradually increased to 1.5 mg. in different series of embryos. There was some indication that the degree of cortical differentiation in duck embryos is conditioned by the increase in estrogen administered,

as found by Wolff and Ginglinger (1935b) and by Willier *et al.* (1937) in chick embryos; but the highest dosage of Progynon administered did not cause the formation of a thick, well-differentiated cortex, comparable to that in the normal female. However, in some of the treated cases receiving the maximum dosage of Progynon a well-developed surface layer was found as thick as the normal cortex in females and resembling that layer before synizesis sets in. The striking feature here, as well as in some other instances noted in this study, was the apparent method by which this "cortex" was being formed. Underlying testicular cords appeared quite obviously to be growing out to the periphery of the gonad, where, by continued development and eventual severance from the underlying cords, they formed or contributed to the "cortical" layer investing the left gonad. Considerable skepticism prevailed as to the validity of applying the term "cortex" to the layer arising in the manner described, particularly in the absence of cells in synizesis, until cases were found in which the gonad showed, all in the same embryo, (1) instances of connecting testicular cords with this outer layer, (2) an area in which this layer was completely detached from the underlying cords, and (3) a patch of fully differentiated cortex.

In reviewing the literature, no reference was found that mentioned such a method of cortex formation. However, it does seem to be in harmony with the principles of development as set forth by Weiss (1939, pp. 320-30) with reference to determination in organ rudiments. Cortex, formed in this manner, may require a somewhat longer period to differentiate fully than when formed from the germinal epithelium, which would account for the absence of cells in synizesis

in many of the cases observed. It should, of course, be emphasized that, in many embryos, cortex was also observed to be developing from the thickened persisting germinal epithelium on the left testis, as observed by others.

The relation between dosage and degree of cortical differentiation was clearly indicated in these experiments. When Progynon failed to cause complete inversion of the testis, diethyl stilbestrol and hexestrol, which were available in higher concentrations, were tried. A very high degree of intersexuality was obtained with the use of these estrogens. The gonads revealed cortex along the posterior portion as thick as that of control females of comparable age. Usually, cortical hypertrophy was more pronounced at the posterior end, with a tendency to become thinner as the anterior end was approached; but cells in synizesis appeared first at the anterior end. This differential in thickening is in keeping with the condition of the persisting epithelium in control males. The difference in degree of response of the duck gonad to Progynon, stilbestrol, or hexestrol is not regarded as attributable to any inherent differences in the action of the hormones but rather to a difference in the dosages used, although we have no data on relative potencies.

In spite of the fact that excellent cortex was obtained following the use of hexestrol, the medulla was apparently qualitatively unchanged as a result of treatment, regardless of the estrogen employed. Forbes (1938), using Progynon on sexually immature alligators, noted that this hormone exercised an effect similar to that observed in these experiments, namely, that there is pronounced cortical hypertrophy but no noticeable effect upon the medulla qualitatively. In addition, he asserts that

there is no significant quantitative change in the medulla, and he interprets this stability as evidence that the estrogen acts by direct stimulation of the cortex instead of by inhibition of the medulla. However, it has not been established indubitably in the duck that there is no volume change in the medullary component of the gonad following estrogen administration, as Forbes claims for the alligator. In the majority of the estrogen-treated males it appears rather certain that there has been no decrease in volume; but in some of the males receiving the highest dosages the difference in total gonad size in favor of the left was immediately evident; however, this disparity could be due to the addition of cortex on the left as well as to reduction of medulla on the right or both. In both testes the medullary component was usually well developed and clearly testicular in type. In Forbes's experiments, cortical differentiation occurred on both testes instead of on the left testis only.

The right gonad in estrogen-treated males usually appeared broader macroscopically than the left, and the latter was, as a rule, longer. In a few of the embryos receiving the highest dosage the right gonad was noticeably smaller than the left, which, as stated above, could be due to the added cortex on the left or to reduction of testicular cord volume or both. Microscopically, the right testis of estrogenized males occasionally showed a few cortical lumps. These were never observed to attain any appreciable thickness, and the frequency with which they were encountered seemed to coincide with the number of cases showing persisting patches of thickened epithelium in controls. There appeared to be no modification of testicular cords, nor could we be certain of a decrease in volume. Willier *et al.* (1937) report no strik-

ing change in the right gonad of estrogenized male chick embryos except in those cases showing extreme modification of the left, in which case the right becomes reduced in size, flattened, and contains medullary tissue. This differs from the condition found in the duck, where the right exhibits a flattening with no medullary tissue, when the left attains merely an intersexual condition.

Several investigators cite evidence to show that estrogens may stimulate cortical development in the female. Dantchakoff (1935b) states that Progynon in the female chick acts like a whiplash in accelerating histogenetic differentiation. Forbes (1938) records hypertrophy of the ovarian cortex of both ovaries in immature female alligators as a result of estrogen treatment. The increase in volume may be as much as 90 per cent.

Macroscopic and histologic study of estrogen-treated female duck embryos reveals an apparent hypertrophy of cortex on the left ovary in some instances, as compared with controls. Too, it was only in estrogen-treated females that a cortex of normal thickness with cells in synzesis was found in the right ovary. It would seem that estrogens exert a specific effect upon the development of cortex in the duck embryo. These results would appear to contradict the observations of Moore and Price (1932) that gonad hormones have no direct effect on the gonads of either the same or the opposite sex; however, it should be noted that their observations were based on experiments carried out upon adult rats, animals with fully developed, mature, and not embryonic reproductive systems. The results obtained in another class of vertebrates where adult or embryonic undifferentiated gonads are subjected to administered sex hormones may, however, not be comparable. It should be noted that

Willier *et al.* (1935, 1936, 1937) observed that in zygotically determined females the right and left ovaries remain usually quite normal in size, form, and histology, regardless of dosage of estrogens administered, whereas in genetic males the testes were reduced in size, though essentially normal in form and structure following administration of testosterone propionate.

The estrogens employed in these experiments apparently have no specific effect upon the Wolffian ducts, but they do have very specific effects upon the Müllerian ducts. This is in keeping with the observations of Willier *et al.* (1935, 1936, 1937), Wolff and Ginglinger (1935a), and Dantchakoff (1936). The fact that greater hypertrophy was obtained with stilbestrol and hexestrol than with Progynon doubtless results from the fact that higher concentrations of the latter were administered. In addition to greater volume, the hexestrol-treated ducts had thicker walls correlated closely with a more abundant glandular development. The degree of hypertrophy of Müllerian ducts was not always an index of the extent of cortical development, for the ducts might show good development and the gonads little or no cortex. Similar observations were made by Willier *et al.* (1937) in the chick. These authors observed that male chicks with completely reversed left gonads had oviducts strikingly similar to those of the normal female. This is in contrast to the modification found in the duck, where low dosage produced almost typical female Müllerian ducts but scant cortical stimulation, whereas the highest dosages, which caused greatest cortical development, occasioned monstrously overdeveloped ducts on both sides.

Willier *et al.* (1937) and Forbes (1938) have observed in the chick and alligator,

respectively, that the most persistent portion of the Müllerian duct in the estrogenized male is the anterior end. This harmonizes with observations in the duck and is in keeping with what might be expected from the manner of normal degeneration of these ducts—from the caudal to the cranial end. Furthermore, in the duck the length of the persistent portions is related to the age at which the embryos receive the hormone. In other words, the earlier the injection, the more nearly normal length the oviduct is likely to be.

The failure of testosterone propionate to exercise any appreciable effect upon the female or male urinogenital system in duck embryos is in contrast to the conclusions of Willier *et al.* (1935, 1936, 1937) with respect to the action of bull-testis extracts upon the chick. Dantchakoff (1937) also observed that testosterone caused an enlargement of the Wolffian ducts in the chick and that, while it was depressing to the cortex and Müllerian ducts and stimulating to the mesonephric tissue, reversal of sex from female to male was not accomplished. She tried at various times to bring about transformation of females to males, having used X-ray irradiation before androgen treatment, which resulted in partial inversion of females (1935b).

Willier, Rawles, and Koch (1938) made an exhaustive study of the effects of male hormones upon the genitourinary system of chick embryos. Here, an effect is noted for bull-testis preparation, which causes hypertrophy of the Wolffian ducts in male and female. Willier *et al.* (1938) also described a masculinizing action of dehydroandrosterone, androstenedione, and testosterone propionate upon genetic females with enormous hypertrophy of the Wolffian ducts.

Wolff (1935a, b, 1936a) recorded a

dual action of androsterone upon chick embryos by which males are feminized and females masculinized; and Wolff and Ginglinger (1936b) demonstrated that certain androgens have an estrogen-like action upon immature female mice, as well as a masculinizing action on combs of capons. Testosterone, which did not show feminizing effects in the experiments of Willier *et al.* (1938) or in the experiments here reported, is included among the androgens mentioned by Wolff and Ginglinger as possessing certain estrogen-like qualities. The possibility that testosterone might possess these qualities was not ruled out by Willier *et al.* (1938) in the light of the observations of Wolff and Wolff (1936), who noted that in two breeds of chickens—the Leghorns, on the one hand, and a cross between Light Sussex females and Rhode Island Red males, on the other—there was a difference in the amount of androgen required to effect the intersexual condition—hence testosterone might cause both masculinization and feminization in other breeds.

The references on work with androgens just cited show how varied the results may be, depending upon the androgen, the experimental animals, and other conditions. However, all seem to agree upon the point that inversion of females is more difficult to bring about in the embryonic stage than inversion of males. This fact, along with the duck's demonstrated resistance to estrogens, may account in part for failure to obtain any demonstrable results with testosterone.

Because of the evident effect of testosterone upon the gonads and Wolffian ducts of the chick as reported by other investigators and because of the apparent absence of an effect observable macroscopically in the duck, histological study of these structures was made. Our study did not give conclusive proof that

this androgen caused any modification in these structures. It is possible, however, that a higher dosage of testosterone propionate than that administered in our experiments might bring about a modification of sexual characters in the duck, since it has proved more resistant, in general, to the hormones administered than the chick, where Willier *et al.* (1938) found that much less than 1.0 mg. of estrogen was sufficient to feminize males completely, while 2 mg. or more of androgen only partially masculinized females. In a recent paper (Moore, 1944) the problem of variations in the effects of hormones and conflicting results obtained by treatment with sex hormones in the various classes of vertebrates is discussed in some detail.

Wolff and Ginglinger (1936a) state that hormones have no effect upon the gonad of the chick if injected after the 8th day of incubation and that only the cortex is modifiable this late in incubation, the medulla having earlier ceased to be responsive. Dantchakoff (1935b) observed that embryos injected on the 4th day showed the greatest modifiability, those injected on the 6th, 7th, and 8th days were intermediate in response, while those injected on the 10th day showed very little effect. Willier *et al.* (1937) link the effective period of hormone action with the interval beginning with sex differentiation, namely, about the 6th day, and terminating with the retrogression of the incipient cortex, about the 11th day.

In the case of the duck, the best cortical development was obtained in embryos injected on the 4th day. Those injected on the 6th day were highly modified but not so fully; however, there were fewer cases in this category. Embryos injected on the 8th day showed a definite falling-off in cortical stimulation, on the

9th day a further drop was evident, and after the 10th day there was no evidence of cortex formation from the germinal epithelium.

According to Wolff (1936b) the Müllerian ducts persist and develop in the male chick if injection occurs before the 10th day. Willier *et al.* (1937) suggest that oviduct response to hormones probably ceases after the 11th day, when retrogression is complete.

Our study of normal development in the duck discloses that the Müllerian ducts complete their retrogression about the 18th day, and it appears that hormone treatment after that date is ineffective in causing hypertrophy of these structures. The whole duct does not show development in the male following later injections, but a segment of the anterior portion, which represents the last part to degenerate, may.

The oviduct in the duck, therefore, seems to agree with that in the chick with reference to the period of modifiability. This period is longer in the duck by 1 week, which appears to coincide with the longer period of incubation. This observation on the oviduct would indicate that the gonad might also be modifiable a week longer in the duck than in the chick. In actuality, the effective period here is not a week longer in keeping with the longer period of embryonic development but agrees with observations on the chick (Dantchakoff, 1935b), namely, from the 4th to the 10th days of incubation. Estrogen treatment after the 10th day causes, in a few cases observed, some testicular cords to push through to the surface, forming large lumps, which resemble those mentioned previously in connection with cortex formation. Embryos injected as late as the 14th day showed this condition. In some individuals injected at earlier stages,

testicular cords were also observed to push to the surface and form a layer in this manner, which showed typical cortical differentiation. Since these cords can be induced by estrogen treatment at 14 days to form this outer layer, it is possible that the layer might differentiate into cortex if allowed to develop a sufficient period. It may be expected that cortical formation of this type tapers off with increase in age at the time of injection, just as it does in cortical development from the germinal epithelium. In other words, a stage is reached when irrevocable determination has been attained by the testicular cords. The time at which this occurs has not been accurately determined, but after the 14th day there was little indication of formation of a surface layer from testicular cords in the manner described.

These observations show that the primary sexual cords may under suitable conditions undergo inversion and form cortical cords and theoretically would indicate that the indifferent gonad, which consists of a primary set of sexual cords, should be capable of transforming in either direction.

VI. SUMMARY

1. The effects of several estrogens and an androgen upon the development of the gonads and gonoducts of the White Pekin duck were studied following injection of these hormones into the incubating egg. As a basis for comparison, normal development was studied beginning from the 9th day of incubation, soon after sex differentiation, to 24 days after hatching.

2. The germinal epithelium of the left testis does not degenerate soon after sex differentiation, as claimed for the chick, but proliferates additional cords, beginning about the 14th day, which become

incorporated into the testis along with the primary cords. The epithelium is found best developed at the posterior end of the gonad. A well-developed germinal epithelium was never noted on the right testis.

3. The Müllerian ducts in the male degenerate posteroanteriorly and have disappeared by about the 18th day. The right oviduct in females degenerates anteroposteriorly, and at 18 days only a posterior vestigial tip remains.

4. The estrogens—alpha-estradiol benzoate (Progynon-B), diethylstilbestrol, and hexestrol—and the androgen—testosterone propionate—were employed. The amount of alpha-estradiol administered varied from 0.25 to 1.50 mg., hexestrol varied from 1.25 to 3.75 mg., and stilbestrol and testosterone were given only in 1.25-mg. quantities.

5. Injections were made from the 3d to the 18th day of incubation, and embryos were killed at from 9 days' incubation to 24 days' post-hatching.

6. Administration of 0.25 mg. of alpha-estradiol in some cases caused the development of a thin cortical layer on the left testis. Doubling the dosage produced slight improvement in cortical development. The highest dosage of alpha-estradiol, 1.50 mg., failed to bring about complete inversion of testes, although cortical development was more pronounced. The right testis was slightly smaller in some of the cases but distinctly testicular, regardless of dosage.

7. Injection of 1.25 mg. of stilbestrol brought about a pronounced intersexual condition in the left testis.

8. Dosages of 1.25–2.50 mg. of hexestrol produced the greatest degree of cortical differentiation observed in males. The cortex at the caudal end of the left testis was as thick as that of the normal female and showed many cells in synize-

sis. The medullary portion of the gonad retained its testicular character. The testicular cords of the right testis showed no modification due to hexestrol treatment. This gonad as a rule was smaller in size.

9. Injection of 3.75 mg. of hexestrol on the 4th–6th days caused death of all embryos by the 10th day of incubation.

10. No definite effect upon the ovaries was noted in any of the estrogen-treated cases.

11. A 1.25-mg. dose of testosterone propionate apparently had no effect upon the gonads or Wolffian ducts of either sex.

12. The lowest dosage of alpha-estradiol usually produced oviducts in males like those of the normal female. The highest dosage caused both oviducts to become greatly hypertrophied. Both oviducts in females were greatly enlarged following injection of the higher dosages of alpha-estradiol, the right persisting in its entirety when injection occurred as late as the 12th day.

13. Treatment with 1.25 mg. of stilbestrol produced considerable hypertrophy of Müllerian ducts in four males and four females, the only survivors of forty-five embryos at 22 days. The high degree of toxicity of this hormone discouraged its further use.

14. With hexestrol treatment in males both Müllerian ducts were usually fully developed when injection occurred on the 4th day and they became so huge as to be grotesque in appearance. The walls were thick and exceedingly glandular. The later in incubation the estrogens were administered, the shorter became the persisting segments of oviduct. These occurred at the anterior end. Oviducts were not usually found when injections were made after the 18th day. Müllerian

ducts in females revealed the same degree of modification as in the male. There was no evident effect of estrogens upon the Wolffian ducts.

15. The greatest degree of reversal was obtained in embryos injected on the 4th day. Those treated on the 5th and 6th days were almost as good, but there was a definite falling-off in degree of cortical development from the 8th to the 10th day. After the 10th day no cortical differentiation was observed to

occur from the germinal epithelium; however, underlying testicular cords were seen pushing through to form a surface layer in embryos injected as late as the 14th day, and in some instances these cords appeared to be differentiating into cortex.

16. Cortical cords may therefore form from the germinal epithelium of the left testis or from primary testicular cords, which have grown out and formed a layer on the surface of the gonad.

LITERATURE CITED

- ALLEN, B. M. 1904. The embryonic development of the ovary and testis of the mammals. *Amer. Jour. Anat.*, **3**:89.
- BENOIT, JACQUES. 1923. A propos du changement expérimental de sexe par ovariectomie chez la poule. *Compt. rend. Soc. biol.*, **89**:1326.
- . 1924. Sur la signification de la glande génitale rudimentaire droite chez la poule. *Compt. rend. Acad. Sci.*, **178**:341.
- . 1926. Etude histologique de la glande génitale droite de la poule ovariectomisée en un testicule du développement. *Ibid.*, **182**:240.
- BRODE, MALCOLM D. 1928. The significance of the asymmetry of the ovaries of the fowl. *Jour. Morphol. and Physiol.*, **46**:1-57.
- BURNS, ROBERT K., JR. 1931. The process of sex transformation in parabiotic *Amblystoma*. II. Transformation from male to female. *Jour. Exper. Zool.*, **60**:339.
- . 1935. The process of sex transformation in parabiotic *Amblystoma*. III. Conversion of testis to ovary in heteroplastic pairs of *A. tigrinum* and *A. punctatum*. *Anat. Rec.*, **63**:101-29.
- BURWELL, LILLIAN L. 1931. Early differentiation of the duck gonad. Master's dissertation, University of Chicago. Pp. 27.
- DANTCHAKOFF, V. 1935a. Sur l'inefficacité de l'hormone mâle à produire une inversion sexuelle des ébauches gonadiques femelles du poulet et sur ses causes. *Compt. rend. Soc. biol.*, **119**:1120.
- . 1935b. Sur l'inversion sexuelle expérimentale de l'ébauche ovarique chez l'embryon du poulet. *Ibid.*, **120**:597.
- . 1935c. Sur les différences de sensibilité des récepteurs tissulaires envers en folliculine, à divers stades embryonnaires. *Compt. rend. Acad. sci.*, **201**:161.
- . 1935d. Sur l'inversion sexuelle expérimentale de l'ébauche testiculaire, chez l'embryon du poulet. *Ibid.*, **200**:1983.
- . 1936. Réalisation du sexe à volonté par inductions hormonales. I. Inversion du sexe dans un embryon génétiquement mâle. *Bull. biol.* **70**:241.
- . 1937. Effets du testostérone-propionate sur les ébauches sexuelles de l'oiseaux. *Compt. rend. Soc. biol.*, **124**:235.
- . 1938. Gewebeplastizität, Hormone und Geschlecht. *Ergebn. d. Physiol.*, **40**:101.
- D'HOLLANDER, F. 1904. Recherches sur l'oogenèse et sur la structure et la signification du noyau vitellin de Balbiani chez les oiseaux. *Arch. d'anat. micr.*, **7**:117-80.
- DOMM, L. V. 1924. Sex reversal following ovariectomy in the fowl. *Proc. Soc. Exper. Biol. and Med.*, **22**:28.
- . 1927. New experiments on ovariectomy and the problem of sex inversion in the fowl. *Jour. Exper. Zool.*, **48**:31.
- . 1929. Spermatogenesis following early ovariectomy in the brown Leghorn fowl. *Arch. f. Entw.-mech.*, **119**:171.
- . 1931. A demonstration of equivalent potencies of right and left testis-like gonads in the ovariectomized fowl. *Anat. Rec.*, **49**:211.
- DOMM, L. V., and JUHN, M. 1927. Compensatory hypertrophy of the testis in brown Leghorns. *Biol. Bull.*, **52**:458.
- FELIX, W. 1912. Chapter xix in KEIBEL and MALL, Human embryology. New York: J. B. Lippincott Co.
- FINLAY, G. F. 1925. Studies on sex differentiation in fowls. *Brit. Jour. Exper. Biol.*, **2**:439.
- FIRKET, JEAN. 1914. Recherches sur l'organogenèse des glandes sexuelles chez les oiseaux. *Arch. de biol.*, **29**:201-352.
- . 1920. Recherches sur l'organogenèse des glandes sexuelles chez les oiseaux. *Ibid.*, **30**:393.
- FORBES, THOMAS R. 1938. Studies on the reproductive system of the alligator. II. The effects of pro-

- longed injection of oestrone in the immature alligator. Jour. Exper. Zööl., **78**:335.
- GOODALE, H. D. 1910. Some results in castration in ducks. Biol. Bull., **20**:35.
- GREEN, R. R., *et al.* 1939. Experimental intersexuality: the effect of antenatal androgens on sexual development of female rats. Amer. Jour. Anat., **65**:415.
- GREENWOOD, A. W. 1925. Gonad grafts in embryonic chicks and their relation to sexual differentiation. Brit. Jour. Exper. Biol., **2**:165.
- GRÜNWALD, P. 1934a. Über Beziehungen zwischen der Beschaffenheit des Hodenepithels und den darunter gelegenen Blutgefäßen. Zeitschr. f. Anat. u. Entw., **102**:424.
- . 1934b. Über Form und Verlauf der Keimstränge bei Embryonen der Säugetiere und des Menschen. *Ibid.*, **103**:278.
- HARGITT, GEORGE T. 1930. The formation of the sex glands and germ cells of mammals. III. Jour. Morphol. and Physiol., **49**:277.
- HETT, J. 1930. Vergleichende Untersuchungen über das persistierende Keimepithel des Hodens einiger Säuger. I. Zeitschr. f. mikr.-anat. Forsch., **20**:185-252.
- . 1932. Vergleichende Untersuchungen über das persistierende Keimepithel des Hodens einiger Säuger. *Ibid.*, **28**:529.
- HOFFMAN, C. K. 1892. Etude sur le développement de l'appareil urogénital des oiseaux. Verh. der Kon. Akad. Wetensch., **1**, Part IV, 1-55.
- HUMPHREY, R. R. 1929. Studies on sex reversal in *Amblystoma*. II. Sex differentiation and modification following orthotopic implantation of a gonadic preprimordium. Jour. Exper. Zööl., **53**:171.
- . 1935. Studies on sex reversal in *Amblystoma*. VII. Reversal of sex type in gonadic preprimordia of *A. punctatum* males implanted in females of more rapidly growing species. Anat. Rec., **62**:223-45.
- KEMP, T. 1927. Studier over Kønsskarakterer Hos fostre. Pp. 1-117. Copenhagen: Levin & Munksgaard.
- KOZELKA, A. W., and GALLAGHER, T. F. 1934. Effect of male hormone extracts, theelin and theelinol, on the chick embryo. Proc. Soc. Exper. Biol. and Med., **31**:1143.
- LAULANÉ, M. 1886. Sur la mode d'évolution et la valeur de l'épithélium germinatif dans le testicule embryonnaire du poulet. Compt. rend. Soc. biol., **38**:87.
- LILLIE, F. R. 1917. The free-martin; a study of the action of sex hormones in the foetal life of cattle. Jour. Exper. Zööl., **23**:371.
- . 1919. The development of the chick. New York: Henry Holt & Co., Pp. 472.
- . 1927. The present status of the problem of "sex-inversion" in the hen: comments on Dr. Domm's paper. Jour. Exper. Zööl., **48**:175.
- MINOURA, T. 1921. A study of testis and ovary grafts on the hen's egg and their effects on the embryos. Jour. Exper. Zööl., Vol. **1**.
- MOORE, C. R. 1944. Gonad hormones and sex differentiation. Amer. Nat., **78**:97-130.
- MOORE, C. R., and PRICE, D. 1932. Gonad hormone functions, and the reciprocal influence between gonads and hypophysis with its bearing on the problem of sex hormone antagonism. Amer. Jour. Anat., **50**:113.
- POPOFF, N. 1908. L'Ovule mâle et le tissu interstitiel du testicule chez les animaux et chez l'homme. Arch. de biol., **24**:433-500.
- RISLEY, PAUL L. 1933. Contributions on the development of the reproductive system in the musk turtle, *Sternotherus odoratus* (Latreille). II. Gonadogenesis and sex differentiation. Zeitschr. f. Zellforsch. u. mikr. Anat., **18**:493.
- ROLNIK, VERA. 1943. Instructions for the incubation of Eider Duck eggs. Jour. Wildlife Management, **7**, No. 2, 155-62.
- SEMON, R. 1887. Die indifferente Anlage der Keimdrüsen beim Hühnchen und ihre Differenzierung zum Hoden. Jener Zeitschr. f. Naturwiss., Vol. **21**:46-86.
- SWIFT, C. H. 1914. Origin and early history of the primordial germ cells in the chick. Amer. Jour. Anat., **15**:483-516.
- . 1915. Origin of the definitive sex-cells in the female chick and their relation to the primordial germ-cells. *Ibid.*, **18**:441-70.
- . 1916. Origin of the sex cords and definitive spermatogonia in the male chick. *Ibid.*, **20**:375-410.
- WEISS, PAUL. 1939. Principles of development. New York: Henry Holt & Co. Pp. 601.
- WILLIER, B. H. 1939. The embryonic development of sex. In Sex and internal secretions, ed. E. ALLEN, p. 64. Baltimore: Williams & Wilkins.
- WILLIER, B. H.; GALLAGHER, T. F.; and KOCH, F. C. 1935. Sex modification in the chick embryo resulting from injections of male and female hormones. Proc. Nat. Acad. Sci., **21**:625.
- . 1936. The action of synthetic male hormones upon the gonads and gonoducts of the chick embryo. Anat. Rec., Suppl., **67**:32.
- . 1937. Modification of sex development in the chick embryo by male and female sex hormones. Physiol. Zööl., **10**:101.
- WILLIER, B. H.; RAWLES, M. E.; and KOCH, F. C. 1938. Biological differences in the action of synthetic male hormones on the differentiation of sex in the chick embryo. Proc. Nat. Acad. Sci., **24**:176.
- WILLIER, B. H., and YUH, E. C. 1928. The problem of sex differentiation in the chick embryo with reference to the effects of gonad and non-gonad grafts. Jour. Exper. Zööl., **52**:65.
- WITSCHI, E. 1927. Sex reversal in parabiotic twins of the American wood-frog. Biol. Bull., **52**:137.
- . 1929. Studies on sex differentiation and sex

- determination in amphibians. I. Development and sexual differentiation of the gonads of *Rana sylvatica*. Jour. Exper. Zool., **52**:235-65.
- . 1934. Genes and inductors of sex differentiation in amphibians. Biol. Rev., **9**:460-88.
- . 1935. Die Amphisexualität der embryonalen Keimdrüsen des Haussperlings *Passer domesticus* (Linnaeus). Biol. Zentralbl., **55**:168.
- WOLFF, ÉTIENNE. 1935a. Sur l'action de l'hormone mâle (androstérone) injectée à l'embryon de poulet: production expérimentale d'intersexes. Compt. rend. Soc. biol., **120**:1312.
- . 1935b. Interprétation des résultats obtenus au cours des expériences d'injection d'androstérone synthétique aux embryons de poulet. *Ibid.*, **120**:1314.
- . 1936a. La double action—masculinisante et féminisante—de l'androstéron sur le tractus génital des embryons de poulet. *Ibid.*, **121**:1474.
- . 1936b. Sur la réaction des canaux de Müller des oiseaux aux hormones sexuelles. *Ibid.*, **123**:237.
- WOLFF, E., and GINGLINGER, A. 1935a. Sur la production expérimentelle d'intersexes par l'injection de folliculine à l'embryon de poulet. Compt. rend. Acad. sci., **200**:2118.
- . 1935b. Caractères des intersexes obtenus expérimentalement chez l'embryon de poulet. *Ibid.*, p. 2221.
- . 1936a. Sur la transformation des poulets mâles en intersexes par injection d'hormone femelle (folliculine) aux embryons. Arch. anat., Histol. et embryol., **20**:279.
- . 1936b. Sur l'action oestrogène de l'androstérone. Compt. rend. Soc. biol., **121**:1476.
- WOLFF, ÉTIENNE, et WOLFF, ÉMILIENNE. 1936. Sur les différences de sensibilité des embryons femelles des deux races de poules à une hormone sexuelle: l'androstérone. Compt. rend. Soc. biol., **123**:1191.

PLATE I

(Initial magnifications reduced by one-half for publication.)

FIG. 16.—Normal left ovary of 22-day embryo. Note cells in synizesis in middle portion and its absence in lateral margins. Initial $\times 120$.

FIG. 17.—Showing enlarged section of cortex from central area of Fig. 16. Initial $\times 450$.

FIG. 18.—Normal left testis of 14-day embryo, showing early formation of cords on surface. Initial $\times 120$.

FIG. 19.—Normal left testis of 18-day embryo. Note continuous layer of cords on surface of gonad. Initial $\times 120$.

FIG. 20.—Normal left testis of 24-day embryo, showing maximum thickening of cords located near posterior end. Initial $\times 120$.

FIG. 21.—Same as Fig. 20 but near middle of testis. Initial $\times 120$.

FIG. 22.—Normal left testis of 28-day embryo, showing group of newly formed cords inside tunica albuginea in close contact with primary cords. Initial $\times 120$.

FIG. 23.—Early stage in formation of cortex from germinal epithelium of left testis. Embryo received 0.50 mg. of Progynon on 4th day and was killed on 18th day. Note underlying testicular cords with inclosed primordial germ cells. Initial $\times 120$.

FIG. 24.—Illustrating differentiation of cortex from germinal epithelium of left testis with lowest dose of Progynon (0.25 mg.). Embryo was injected on 3d day. Age 25 days. Note typical testicular cords underlying cortical layer. Initial $\times 90$.

PLATE II

(Initial magnifications reduced by one-half for publication.)

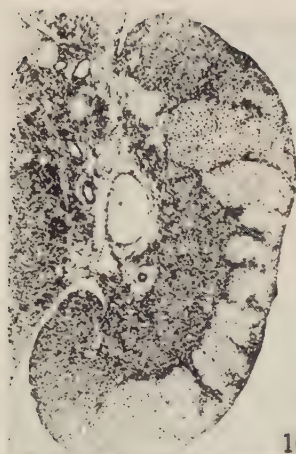
FIG. 25.—Illustrates the maximum development of cortex observed. This section was taken from the left gonad of the embryo shown in Fig. 13. It received 1.25 mg. of hexestrol on the 4th day. Age 26 days. Note testicular character of medullary area underlying cortical layer. Initial $\times 120$.

FIG. 26.—Higher magnification of a portion of cortical layer shown in Fig. 25. Initial $\times 180$.

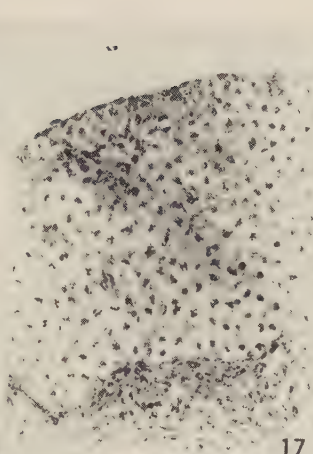
FIG. 27.—From left gonad in Fig. 25, showing relation between surface cortex and underlying testicular cords. Initial $\times 120$.

FIGS. 28, 29, and 30.—These sections are from the same embryo and reveal stages in the formation of differentiated cortex from primary cords of the left testis. Fig. 28, outgrowing cords; Fig. 29, surface layer cut off from the underlying tubules; and Fig. 30, patch of dif-

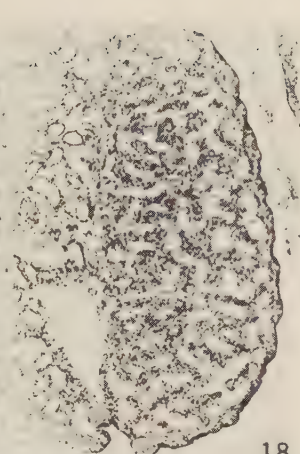
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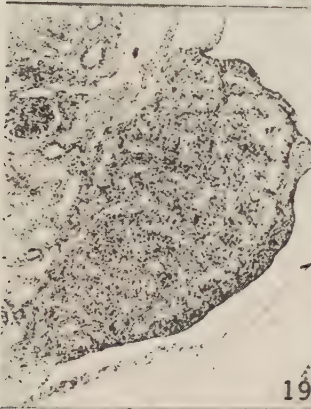
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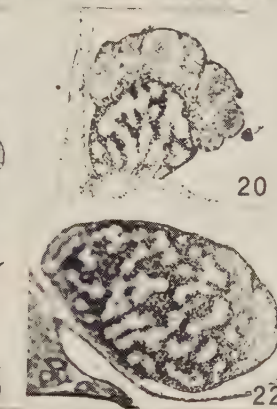
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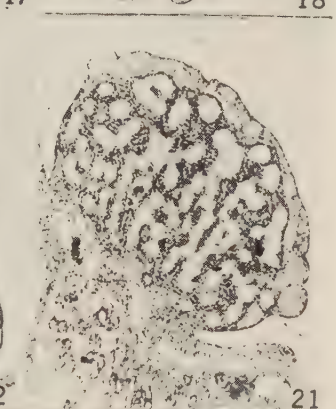


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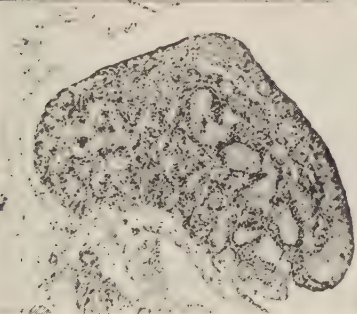


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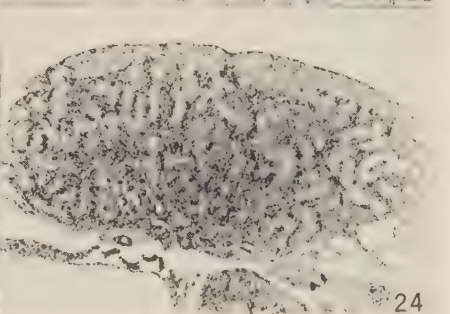
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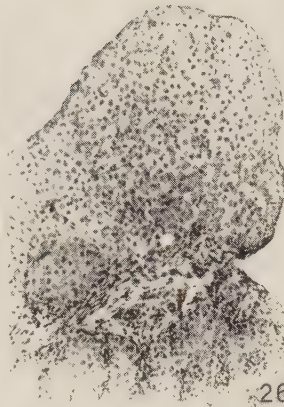


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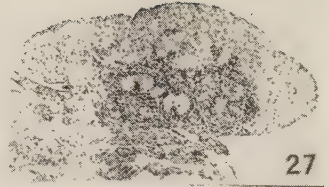
PLATE II



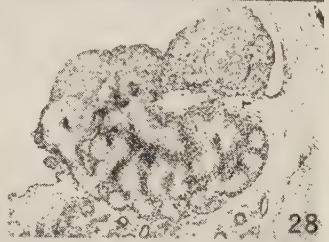
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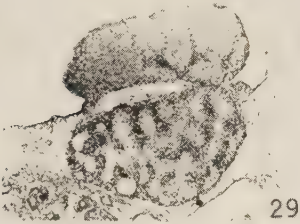
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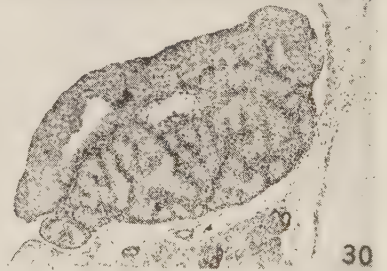
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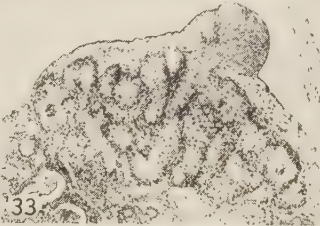
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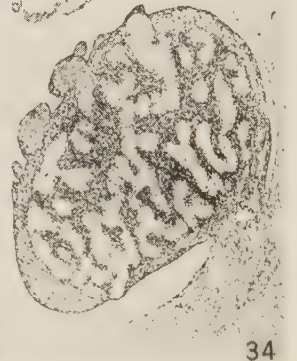
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ferentiated cortical cells in the surface layer. The embryo received 1.0 mg. of Progynon on the 6th day and was killed when 26 days old. Initial $\times 120$.

FIG. 31.—Illustrates an early stage in outgrowth of testicular cords forming surface lumps. Embryo received 0.25 mg. of Progynon on 4th day and was killed on 22d day. Initial $\times 120$.

FIG. 32.—Shows tubule of left testis connected with surface cap of cells. This layer becomes differentiated into cortex toward the

anterior end of gonad. Embryo received 0.50 mg. of Progynon on 4th day and was killed on 25th day. Initial $\times 120$.

FIG. 33.—Shows outgrowth of primary cords to surface, where differentiation follows. This embryo received 1.0 mg. of Progynon on 6th day. Age 22 days. Initial $\times 120$.

FIG. 34.—Testicular cords of left gonad pushing to surface, following injection of 2.50 mg. hexestrol on 14th day. Embryo killed on the 28th day. Initial $\times 120$.

